BULLETIN
OF
THE JOHNS HOPKINS HOSPITAL

(THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL)

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VOLUME XCVI

BALTIMORE
THE JOHNS HOPKINS PRESS
1955

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VOLUME 96 NUMBER 1

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

UNIVERSITY OF MICHIGAN
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January, 1955

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PUBLISHED MONTHLY
THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland
Made in United States of America

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Books for review should be sent to The Editor at the above address.

Correspondence concerning business matters and all remittances should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

Subscription price. In the United States and its possession $8.00 per year. In Canada $8.25 per year; in foreign countries $8.50 per year. Single numbers, $1.00.

New subscriptions and renewals are entered to begin with the first issue of the current year. Subscribers in the United States are hereby notified that missing numbers cannot be replaced unless notification of loss is received in this office within 1 month following publication of the missing number; foreign subscribers 6 months.

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THE JOHNS HOPKINS PRESS

BALTIMORE-18, MARYLAND, U. S. A.

Entered as second class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

Medical

IN VITRO CALCIFICATION OF RACHITIC RAT CARTILAGE IN NORMAL AND PATHOLOGICAL HUMAN SERA WITH SOME OBSERVATIONS ON THE PATHOGENESIS OF RENAL RICKETS

EDMUND R. YENDT*, THOMAS B. CONNOR AND JOHN EAGER HOWARD

The Department of Medicine, The Johns Hopkins University School of Medicine and Hospital

Received for publication September 21, 1954

INTRODUCTION

Bone is a tissue composed of cells and intercellular substance in which are deposited crystalline lime salts. During periods of growth, the cartilage at the epiphyses goes through a phase in which its intercellular substance is calcified in a similar manner. In the disease state rickets, irregular arrangement of the cartilage cells occurs at the epiphyseal line and the cartilaginous matrix fails to calcify. Also, in rickets, osteoid borders of uncalcified matrix are much wider than in the normal. An abnormally low concentration of calcium, inorganic phosphate or both in the internal environment—i.e., the extracellular fluids—has been shown to be an important factor in the failure of cartilage and bone matrices to calcify in rickets. Howland (1) observed that in babies, these features of rickets were not seen pathologically unless the product† of calcium and phosphorus‡ in the child's blood was considerably less than normal, and Shipley showed that rachitic cartilage would calcify in vitro if placed in normal baby's serum (2a) or in artificial solutions containing calcium and phosphate in concentrations equivalent to those of normal extracellular fluid (2b).

In rats made rachitic by means of inadequate diets, there was found the same relationship between the concentration of calcium and phosphorus in the serum and the presence or absence of calcification of the cartilage matrix and osteoid. Extensive studies on the vagaries of in vitro calcification of the cartilage of the rachitic rat have been made, using both sera and artificial solutions of salts; and from these have resulted many deductions regarding the mechanisms of skeletal calcification. However, there are still points about these mechanisms which remain obscure.

* The studies herein reported were carried out during the tenure of a travelling fellowship from the R. Samuel McLaughlin Foundation. Dr. Yendt is currently at the Toronto General Hospital, Toronto, Canada.

† The term Ca × P product is commonly used to denote the product of the calcium concentration expressed as mg. per 100 ml. × the inorganic phosphorus concentration expressed as mg. per 100 ml.

‡ The terms "serum phosphorus" and "ultrafilterable phosphorus" are used throughout this paper to refer to the inorganic phosphorus of the serum and of the serum ultrafiltrate respectively.

The object of the present experimental study was to throw light on three major points:

1. By using sera in which there were alterations in calcium or phosphorus content due to a variety of different disorders, to determine if these elements alone are important in the *environmental* component of calcification.

2. To see if the anatomical lesions of rickets seen so frequently in patients with chronic renal insufficiency are due primarily to an abnormality of the quality of the matrix or to something circulating in the body fluids which prevents calcification despite concentrations of calcium and phosphorus which would normally result in good calcification.

3. To determine whether evidence derived from this technique favors the thesis that extracellular fluid contains approximately one-half as much calcium (3) as does serum or, more nearly, 70% as much (4).

METHODS

The technique of in vitro calcification used in this study was similar to that originally described by Shipley, Kramer and Howland (5). Male Wistar rats* three to four weeks old and weighing 40 to 55 grams were fed a high calcium, low phosphorus rachitogenic diet (Rachitogenic diet #2 U.S.P. XII†) and distilled water for a period of 20 to 30 days. At the end of this time, when moderately rachitic, the animals were sacrificed by a blow on the head. Using sterile technique, the upper ends of the tibiae were removed and dissected free of soft tissue. With a razor blade, longitudinal slices approximately 0.75 to 1.0 mm. in thickness were then cut free-hand through these bones, so that each slice included epiphysis, the epiphyseal cartilage, metaphysis and a little of the shaft. Care was taken not to make these slices too thin, since we have confirmed the reports of earlier workers (5) that, for some unknown reason, the calcifiability‡ of such slices is reduced. One or two slices from each rat were used as controls and treated only with silver nitrate solution. If the zone of epiphyseal cartilage was too narrow because the rats were not sufficiently rachitic or if any calcification was present in the epiphyseal cartilage indicating that healing had begun in vivo, results were difficult to interpret. Slices from such animals were not used in the study.

As soon as the slices were cut, they were immediately placed in 50 ml. Erlenmeyer flasks containing 5–10 ml. sterile serum or 10 ml. of a previously prepared solution of calcium, inorganic phosphate and other electrolytes in concentrations approaching those of normal human serum. The importance of placing these slices in the appropriate sera or inorganic solutions as quickly as possible needs emphasis. If they are first allowed to stand at room temperature for as little as 30 minutes, their calcifiability is reduced. After the slices had been placed in the sera or inorganic solutions, pH was adjusted to 7.30 to 7.50 by shaking with 5% CO_2 in 95% O_2 for 3 to 4 minutes. The flasks were then stoppered and mechanically shaken in the incubator at 37.5°C for 18 to 24 hours.

Following incubation, the pH of the contents of each flask was determined. The pH of most solutions and sera was between 7.3 and 7.5 and was never outside the range 7.2 to

* Obtained from Research Supply Company, Philadelphia.

† Obtained from Nutritional Biochemical Corporation, Cleveland, Ohio.

‡ Calcifiability is probably not a good word but it is used widely throughout the literature on "in vitro" calcification. It is used in this communication for the sake of convenience to denote the potential ability of the cartilage to become calcified.

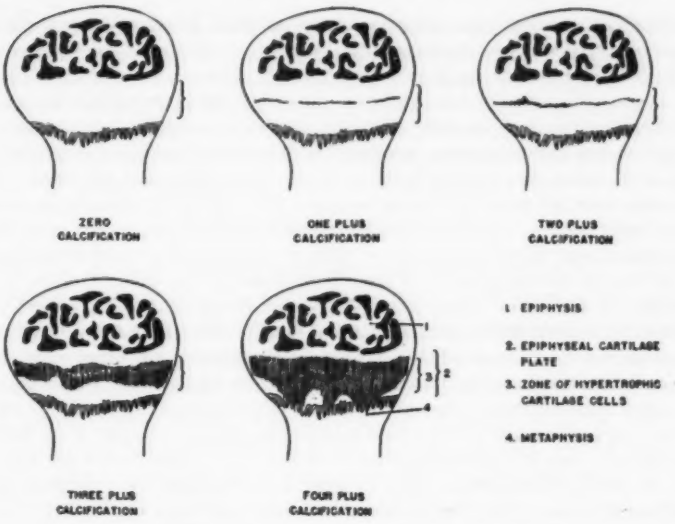
ZERO CALCIFICATION
ONE PLUS CALCIFICATION
TWO PLUS CALCIFICATION
1. EPIPHYSIS
2. EPIPHYSEAL CARTILAGE PLATE
3. ZONE OF HYPERTROPHIC CARTILAGE CELLS
4. METAPHYSIS
THREE PLUS CALCIFICATION
FOUR PLUS CALCIFICATION
METHOD FOR GRADING DEGREE OF CALCIFICATION IN VITRO OF RACHITIC RAT BONE SLICE

FIG. 1. Diagram modelled after that of Marks, P. A., Hiatt, H. H. and Shorr, E.: Factors influencing deposition of calcium and strontium in cartilage in vitro. J. Biol. Chem., 1953, **204:** 177.

7.6. Variations within this pH range do not influence in vitro calcification (5, 6, 7). The slices were then fixed first in 95% and next in absolute ethyl alcohol for 12 to 24 hours. They were then immersed for 15 to 30 minutes in a 1% solution of silver nitrate in bright daylight, but not in direct sunlight. After this, the slices were rinsed in distilled water and allowed to remain in water for several hours until all the blackening that was going to occur had taken place. They were then dehydrated in alcohol, cleared in xylol, mounted and examined under the low power microscope. The degree of calcification was graded roughly 0 to 4+ (Fig. 1).

In the majority of these experiments, sterile technique was used throughout. The necessity for this was emphasized by earlier workers (5, 8). It has since been suggested that sterile technique is unnecessary, at least when inorganic solutions are used. However, in experiments involving the use of unsterile sera, we have found that gross bacterial contamination has occurred by the end of 18 to 24 hours. This often results in a reduction of pH from 7.4 to as low as 6.8. Although most calcification probably occurs in the first 8 to 12 hours, before bacterial growth has resulted in marked pH changes, we have preferred to eliminate this factor by using sterile technique.

Some sera had been frozen at −20°C for periods varying from a few days to one or two years before being used in the experimental procedure. This did not appear to alter the calcifying ability of the sera. Nearly all sera were rendered free of bacteria by passage through an ultra-fine sintered glass filter (Corning #36060). In a few cases, however, blood was drawn and serum separated using sterile technique, making passage through this filter unnecessary. Only six sera were not sterile. The lack of sterility is in each case indicated in the charts. Serum calcium, phosphorus and alkaline phosphatase determinations were always made on serum drawn at the same time as that used for the in vitro calcification studies. Magnesium

determinations were also done on many of these specimens. In most instances, ultrafilterable calcium and phosphorus determinations were carried out on the same samples of sera using the Lavietes (9) technique as described by Hopkins, Howard and Eisenberg (4). In some cases, however, the ultrafiltration studies were made on serum drawn from the same patient on a different date. In these latter instances, the values obtained for *percentage* of ultrafilterable calcium and phosphorus were used in determining the levels of ultrafilterable Ca and P of the serum that was employed in the in vitro calcification procedure. Ultrafiltration studies were not performed on the sera of a few patients. In these latter cases, serum proteins being normal, values for ultrafilterable calcium and phosphorus were obtained on the following basis: ultrafilterable Ca = 68% of total serum Ca and ultrafilterable P = 113% of total serum inorganic* P. These figures represent the average values obtained by this method in a group of normal sera (10). The percentage of the total Ca and P which is ultrafilterable in most pathological sera is also usually within normal range, so long as the serum proteins are not abnormal (10). Usually the concentrations of other major electrolytes were also determined on serum obtained the same day as that used for the calcification studies. In a few instances these values were obtained one to several days sooner or later.

It has long been recognized that factors other than the constituents of the bathing solutions play some part in determining whether or not the cartilage from a particular rat will or will not calcify. There is some slight variation in the calcifiability of cartilage from different rachitic rats even when all experimental conditions are kept identical (6). In addition, factors such as the rate of growth while on the rachitogenic diet (11), the total period of time on the diet (11), and whether or not the rat has been starved just prior to sacrifice (5) have been reported to affect the calcifiability of the cartilage. This variation in the calcifiability of rachitic rat cartilage can be ascertained by determining the lowest Ca × P product† which will calcify the cartilage in vitro. Because of these variables, the later experiments (approximately one-half the total) were controlled by determining the lowest Ca × P product in inorganic solutions which would calcify cartilage from the particular rat used.

The control experiments were performed in the following manner. Two or three of the cartilage slices obtained from each rat were incubated in sera. The remaining slices were incubated in a series of inorganic solutions in which the Ca × P products were 22.5, 25.0, 27.5, 30.0, 32.5 and 35.0. In each of these solutions, the inorganic phosphorus content was kept constant at 5.0 mg. per 100 ml. The calcium concentration was usually 4.5 mg. per 100 ml. in the first flask and was increased by 0.5 mg. per 100 ml. increments in each successive flask so that the last one contained 7.0 mg. per 100 ml. These solutions also contained sodium, potassium, magnesium, chloride, bicarbonate and sulphate ions in approximately the same concentrations as are present in normal serum.‡

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* This value results from the Donnan equilibrium effect on a wholly ultrafiltrable anion.

† In inorganic solutions, the *total* concentrations of calcium and inorganic phosphorus were used to determine the Ca × P product. In serum, the calcium bound to protein is not biologically active and, therefore, the concentrations of calcium and inorganic phosphorus in the serum ultrafiltrate were used to calculate this product.

‡ Control solutions were prepared from the following stock solutions: (1) Double strength basal solution containing NaCl 12.0 gm. per litre, $NaHCO_3$ 4.4 gm. per litre, KCl 0.6 gm. per litre and $MgSO_4 \cdot 7\ H_2O$ 0.5 gm. per litre. (2) Solution of $NaKHPO_4$ (P concentration = 50 mg. per 100 ml.). (3) Solution of $CaCl_2$ (Ca concentration = 70 mg. per 100 ml.). All stock solutions were sterilized by passage through an ultra-fine sintered glass filter (Corning #36060). All control solutions had a final volume of 10 ml. Each was prepared by taking 5.0 ml. of double strength basal solution and then calculating the volumes of $NaKHPO_4$

Serum calcium determinations were made in duplicate by the Clarke-Collip (12) modification of the Tisdall method. Phosphorus analyses were done by the Umbreit (13) modification of the Fiske-Subbarow (14) method. Alkaline phosphatase determinations were made by the Bodansky method (15). Magnesium was determined as magnesium phosphate by the method of Denis (16) modified in that the phosphate was determined by Umbreit's method. The Beckman pH meter (Model G) was used for pH determinations. The pH readings were made at room temperature (25–28°C) and a temperature correction factor was not applied.

RESULTS

Rachitic rat cartilage was incubated in a total of 58 human sera. These sera were obtained from patients in whom there was no obvious derangement of calcium or phosphorus metabolism and from other patients with a variety of disease states in whom abnormalities in the concentration of serum calcium and phosphorus were present. On analyzing the results, it soon became apparent that calcification occurred with lower Ca × P products in the sera of patients who did *not* have renal insufficiency. Because of this, the patients have been divided into three groups: 1. Those without renal insufficiency; 2. Those with chronic renal insufficiency; 3. Those with relatively acute and mild renal insufficiency usually associated with, and possibly secondary to, hypercalcemia.

The results using sera from 30 patients who did not have renal insufficiency are given in Table I. In 11 cases, the Ca × P product of the serum ultrafiltrate was 33 or less. None of these sera calcified rachitic rat cartilage. In the remaining 19 cases, the Ca × P product was 34 or greater. All but two of these sera produced calcification. The two exceptions had Ca × P products of 36 and 37. It will be noted that in some instances a Ca × P product of 34 or more was achieved by virtue of an elevated serum calcium and in others by virtue of an elevated serum phosphorus, but that calcification occurred in either case.

The results obtained using sera from 19 patients who had chronic renal insufficiency are given in Table II. In all but one of these cases, the Ca × P product of the ultrafiltrate was 35 or more. Hence if these sera had behaved as sera from patients without renal disease, all or nearly all would have been expected to produce in vitro calcification. However, calcification occurred in only eight instances, and usually not unless the Ca × P product of the serum ultrafiltrate was over 55. In these cases there was no constant relationship between the presence or absence of calcification and the degree of renal insufficiency as judged by the serum non-protein nitrogen, although in the two instances where calcification did occur with Ca × P products of less than 55, the

and $CaCl_2$ solutions required to give the desired concentrations of calcium and phosphorus and the volume of sterile distilled water necessary to bring the final volume to 10 ml. The phosphate solution and distilled water were always added to the double strength basal solution first. The cartilage slices were then added and the pH adjusted. Calcium solution was added last, after the pH had been adjusted, to avoid the formation of a calcium phosphate precipitate.

TABLE I

In Vitro Calcification of Rachitic Rat Cartilage Using Serum from Patients Who Did Not Have Chronic Renal Insufficiency

Patient	J.H.H. History No.	Diagnosis	Date	Serum								Ultrafiltrate			Controls[a]	Results (Calcification)
				Ca mg.%	P mg.%	Alk. p'tase Bod. U.	Mg mg.%	NPN mg.%	T.P. gm.%	Alb. gm.%	Glob. gm.%	Ca mg.%	P mg.%	Ca × P		
W.B.	555718	Carcinoma of prostate	12–27–52	8.4	2.5	58.0	2.9	45	6.6	—	—	6.5	2.8	18	—	0
L.L.	515221	Hypoparathyroidism, post-thyroidectomy	1–26–54	5.0	7.2	4.1	1.5	36	7.2	4.3	2.9	3.2	8.3	27	27.5	0
J.C.	476862	Renal calculus	2–18–53	10.7	3.1	5.2	2.2	29	6.9	5.1	1.8	7.8	3.4	27	—	0
T.C.	—	Normal		11.0	3.3			35	7.3			7.7[b]	3.7[b]	28[b]	—	0
W.S.	438562	Parathyroid adenoma	12–22–52	14.2	2.8	3.6	2.1	37	7.1	5.0	2.1	9.5	3.0	29	—	0
H.T.[c]	604014	Parathyroid adenoma	9–28–53	11.5	3.3	19.0	2.6	40	6.2	3.9	2.3	7.0	—	—	30.0	0
T.T.	112986	Hypoparathyoidism, post-thyroidectomy	1–21–54	9.1	4.3	3.9	2.0	24	7.5	5.4	2.1	6.1	5.0	31	25.0	0
A.C.	229986	Idiopathic hypoparathyroidism	11–27–53	9.1	4.2	3.9	2.3	27	6.2	4.6	1.6	6.8	4.6	31	25.0	0
M.F.	534975	Hypoparathyroidism; Vitamin D intoxication	5–4–54	13.0	3.2	4.3	3.1	43	7.1	4.6	2.5	8.7	3.6	31	22.5	0
B.F.	661604	Parathyroid adenoma	2–4–54	18.2	1.9	11.2	2.0	40	7.1	5.2	1.9	12.4	2.6	32	27.5	0
N.C.	612588	Acromegaly	11–13–53	10.6	4.0	5.3	2.0	21	7.4	4.4	3.0	7.4	4.4	33	32.5	0
W.S.	438562	Parathyroid adenoma	1–16–53	15.5	3.1	3.3	1.6	27	5.8	—	—	10.3[b]	3.3[b]	34[b]	—	+++
A.M.	256843	Hypoparathyroidism, post-thyroidectomy	10–31–53	8.4	5.5	5.2	—	23[d]	7.6	4.8	2.8	5.7	5.9	34	35.0	+++
E.M.	578188	Bronchogenic carcinoma with bone metastases	2–2–54	13.8	4.0	7.3	1.8	35	6.4	—	—	8.7	4.1	36	25.0	++++
E.P.	602354	Hypoparathyroidism, post-thyroidectomy	6–8–53	9.9	4.8	3.1	—	23	7.1	—	—	6.9[b]	5.3[b]	36[b]	25.0	+++
M.F.	639718	Hypoparathyroidism; A.T. 10 intoxication	5–19–53	13.6	3.5	4.5	2.4	41	6.4	4.4	2.0	9.7	3.7	36	—	++
J.H.	656227	Hodgkin's disease	11–13–53	14.4	3.4	7.6	2.7	29	6.3	3.9	2.4	9.9	3.7	37	25.0	0
C.K.	630985	Carcinoma of esophagus with bone metastases	11–5–53	13.9	3.5	7.2	2.3	35	7.2	3.9	3.3	9.7	3.9	38	30.0	+++
B.G.	554996	Hypoparathyroidism, post-thyroidectomy	6–1–53	9.9	4.7	4.5	—	27	7.1	4.9	2.2	7.2[b]	5.5[b]	39[b]	27.5	+
		Pooled adult serum # 51		10.7	4.7	—			7.2	—	—	7.3[e]	5.3[e]	39[e]	—	++
M.F.	534975	Hypoparathyroidism; vitamin D intoxication	5–11–54	15.8	3.8	—	2.6	45	6.9	—	—	9.1[b]	4.3[b]	39[b]	25.0	+++
D.I.	515253	Anorexia nervosa	1–18–54	10.6	4.9	4.9	1.7	32	7.3	4.5	2.8	7.6	5.5	42	25.0	+++
		Pooled child serum C		9.8	5.6							8.0	5.8	46	—	+++

B.R.	554152	Cushing's disease, post-adrenalectomy	10–31–53	9.7	5.0	5.1	2.0	33	6.2	4.2	2.0	7.7	5.7	44	25.0	+++
R.L.	623117	Renal calculi (Blood drawn during I.V. Ca administration)	12–6–52	14.2	4.3	5.4	2.5	37	7.0	5.2	1.8	9.7[e]	4.7[e]	46[e]	—	+++
		Pooled child serum B		10.2	6.0		2.8					7.0	6.6	46	—	+++
A.M.	256843	Hypoparathyroidism, post-thyroidectomy	10–24–53	10.8	7.0	5.7	2.1	26[d]	8.3	4.4	3.9	6.8	6.9	47	30.0	+++
A.M.	256843	Hypoparathyroidism, post-thyroidectomy	5–20–53	8.6	6.8	3.5	2.1	35	7.7	—	—	6.3[b]	7.5[b]	47[b]	—	+++
		Pooled adult serum A		10.9	4.2							8.1	4.4	36	—	0
W.C.	383713	Multiple myeloma	1–8–54	13.9	5.3	5.3	2.5	45	7.0	5.4	1.6	10.6	5.8	61	—	+++

[a] The figures in this column represent the lowest Ca × P products of control inorganic solutions in which cartilage from the same rat calcified.

[b] Ultrafiltration studies made on serum drawn from same patient on a different date. The values thus obtained for *percentage* of ultrafilterable Ca & P were used in determining the values for ultrafilterable Ca × P in the serum used for in vitro calcification.

[c] Serum not sterile.

[d] N.P.N. determined on serum.

[e] Sera from these patients never ultrafiltered. Ultrafilterable Ca calculated as 68% total serum Ca. Ultrafilterable P calculated as 113% total serum inorganic P.

TABLE II

In Vitro Calcification of Rachitic Rat Cartilage Using Serum from Patients Who Had Chronic Renal Insufficiency

Patient	J.H.H. History No.	Diagnosis	Date	Serum												Ultrafiltrate			Controls[a]	Results (Calcification)
				Ca mg. %	P mg. %	Alk. p'tase Bod. U.	Mg mg.%	Na meq/L	K meq/L	CO_2 c.p. meq/L	Cl meq/L	NPN mg.%	T.P. gm.%	Alb. gm.%	Glob. gm.%	Ca mg. %	P mg. %	Ca × P		
I.R.[b]	636060	Polycystic kidneys	4–30–53	10.3	4.0	4.9	2.0	134	4.6	16.6	106.8	132	6.8	—	—	6.4	4.4	25	27.5	0
R.W.	295760	Chronic glomerulonephritis	12–31–53	9.3	4.4	6.2	4.5	131	—	9.9	100.2	190	5.4	3.6	1.9	6.5	4.8	31	—	0
M.D.	274469	Hypertensive cardiovascular disease. Nephrosclerosis.	2–3–53	7.0	6.4	4.2	4.5	120	5.2	16.6	90.5	100	6.3	3.4	2.9	4.9[c]	7.0[c]	35[c]	—	0
H.S.	632315	Hypertensive cardiovascular disease. Nephrosclerosis.	3–18–53	10.3	4.2	1.6	2.7	136	5.2	24.2	100.4	49	6.9	3.9	3.0	8.5	4.6	39	—	0
M.F.	124368	Chronic pyelonephritis	12–20–52	8.7	5.6	3.4	2.6	147	6.6	8.2	119.3	228	7.0	4.0	3.0	6.3	6.4	40	—	0
B.W.	662123	Hypertensive cardiovascular disease. Nephrosclerosis	2–1–54	10.2	7.0	5.8	4.4	125	3.7	28.4	87.6	80	7.7	4.7	3.0	6.0	6.9	41	22.5	++++
J.M.	287704	Chronic pyelonephritis	3–16–53	8.1	5.1	7.4	2.5	130	3.7	19.9	94.1	62	5.9	3.5	2.4	7.5	5.6	42	—	0
C.R.	198446	Chronic pyelonephritis	11–15–52	10.2	4.8	3.6	4.0	—	—	22.8	98.0	100	—	—	—	7.6[d]	5.7[d]	43[d]	30.0	0
L.W.	240496	Disseminated lupus erythematosus	10–21–53	8.2	5.6	5.1	2.5	141	4.7	21.6	100.0	86	6.0	2.6	3.4	7.1	6.3	45	35.0	0
E.S.	473398	Chronic glomerulonephritis. Nephrotic syndrome	10–24–53	8.5	6.9	6.7	2.9	122	5.0	19.9	100.0	69	2.8	0.9	1.8	6.9	7.4	51	37.5	+
E.W.	658808	Hypertensive cardiovascular disease. Nephrosclerosis.	12–23–53	7.9	6.9	21.4	4.7	119	6.8	24.2	89.8	150	6.0	3.3	2.7	6.3	8.1	51	30.0	0

		Nephrosclerosis.																	
G.B.	167050	Hypertensive cardiovascular disease. Nephrosclerosis.	3–18–53	10.1	6.8	—	4.8	127	5.4	26.2	79.2	196	6.5	—	—	7.1[e]	7.5[e]	53[e]	—	0
B.M.	391302	Chronic glomerulonephritis	2–17–53	8.6	8.0	4.3	3.2	128	4.4	13.2	99.5	154	6.6	4.3	2.3	6.1	9.1	55	—	+++
A.B.	153465	Hypertensive cardiovascular disease. Nephrosclerosis	1–14–53	9.7	7.8	—	3.2	130	3.3	14.4	103.4	113	6.3	4.8	1.5	6.8[e]	8.6[e]	58[e]	—	++++
M.T.	615182	Hypertensive cardiovascular disease. Nephrosclerosis	2–11–53	6.8	10.4	4.0	2.9	121	5.4	10.7	94.5	160	6.7	3.3	3.4	5.5	11.6	64	—	+++
C.M.	396421	Hypertensive cardiovascular disease. Nephrosclerosis	2–3–53	9.0	8.9	4.9	7.9	113	5.2	23.3	76.0	218	7.4	4.7	2.7	6.8	10.3	70	—	+++
C.C.	630487	Chronic glomerulonephritis; secondary hyperparathyroidism	2–3–53	10.2	9.5	12.5	2.3	—	—	14.9	106.0	154	5.9	3.9	2.0	7.4	10.3	76	—	+++
L.K.		Chronic nephritis	7–27–53	9.1	11.2	14.0	—	—	—	16.6	101.0	176	5.4	3.0	2.4	6.8	12.8	87	25.0	0
C.R.	198446	Chronic pyelonephritis	9–13–53	8.8	12.8	3.6	4.0	140	5.8	24.6	108.0	160	6.5	—	—	6.6	15.2	100	—	++++

[a] The figures in this column represent the lowest Ca x P products of control inorganic solutions in which cartilage from the same rat calcified.

[b] Serum not sterile.

[c] Serum from these patients never ultrafiltered. Ultrafilterable Ca calculated as 68% total serum calcium. Ultrafilterable P calculated as 113% total serum inorganic P.

[d] Ultrafiltration studies made on serum drawn from same patient on a different date. The values thus obtained for *percentage* of ultrafiltrable Ca & P were used in determining the values for ultrafilterable Ca & P in the serum used for in vitro calcification.

degree of renal insufficiency was relatively mild (cases B. W. and E. S.). The possible significance of these findings will be discussed later.

The results using sera from an additional 10 patients who had renal insufficiency which was relatively mild and of relatively short duration are given in Table III. These patients formed a rather special group in that for the most part hypercalcemia was present also. One exception was G. G. who had mild renal insufficiency due to multiple myeloma without hypercalcemia. The behavior of this group fell somewhere between those in Table I and those in Table II. Calcification did not occur in sera from J. E., R. H. and H. T. with ultrafiltrate Ca × P products of 37, 38 and 51, respectively. These products would ordinarily be high enough to produce calcification. On the other hand, the degree of inhibition of calcification did not appear to be so marked as in patients with chronic renal disease, because calcification occurred in the sera of patients F. T., J. C. and M. J. when the ultrafiltrate Ca × P products were 43, 44, 47 and 49. In patients with chronic renal disease calcification usually did not occur with products this low. In the case of J. C., good calcification occurred with a Ca × P product of 44 when the serum non-protein nitrogen was 49 mg. per 100 ml. but just a slight degree of calcification occurred when the serum non-protein nitrogen was 67 mg. % in spite of a higher Ca × P product of 47. The case of G. G. is of special interest. Ordinarily when the *total* serum calcium and phosphorus are within normal limits, as was the case here, the serum of the adult human does not calcify rachitic rat cartilage. However, it was expected that calcification might occur in this instance because of a very low serum albumen of 1.9 gm. per 100 ml. which would probably allow a greater proportion of the serum calcium to be ultrafilterable (10). Calcification did indeed occur in this serum but, surprisingly, only 47% of the total serum calcium was found to be ultrafilterable and this resulted in a very low Ca × P product of 17 in the ultrafiltrate. A possible explanation for this apparent discrepancy is the fact that this patient's serum contained a cryoglobulin which resulted in its becoming completely solidified at refrigerator temperature (5°C). In vitro calcification studies were carried out at 37.5°C whereas ultrafiltration studies were performed at room temperature (25°C). If the ultrafiltration studies had been done at 37.5°C, a much higher percentage of the serum calcium would perhaps have been ultrafilterable.

Because the presence of magnesium is known to have an inhibitory effect upon calcification in vitro, (6, 17, 18, 19) magnesium levels were determined on a number of the sera in this study. In our laboratory, the normal range for serum magnesium is 1.8–2.8 mg. per 100 ml. Magnesium determinations were made in 23 patients who did not have renal insufficiency and values at or below 2.8 mg. per 100 ml. were obtained in all but two cases (Table I). Determinations were made in 18 patients with chronic renal disease and in all but six, the values were over 2.8 mg. per 100 ml. (Table II). In eight of these cases, the serum

TABLE III

In Vitro Calcification of Rachitic Rat Cartilage Using Serum from Patients Who Had Renal Insufficiency Which Was Not Primarily Due to Chronic Renal Disease

Patient	J.H.H. History No.	Diagnosis	Date	Serum							Ultrafiltrate			Controls[a]	Results (Calcification)
				Ca mg.%	P mg.%	Alk. p'tase Bod. U.	Mg mg.%	NPN mg.%	T.P. gm.%	Alb. gm.%	Glob. gm.%	Ca mg.%	P mg.%	Ca × P		
G.G.[b]	641861	Multiple myeloma	6–12–53	10.7	3.9	3.4		58	13.4	1.9	11.5	5.0	3.4	17	27.5	++
J.E.[b]	494297	Sarcoidosis	1–22–51	13.2	3.6	4.3	1.6	75	7.9	—	—	9.5[c]	3.9[c]	37[c]	30.0	0
R.H.	610287	Sarcoidosis	1–4–53	13.6	3.8	2.8	2.7	62	8.2	5.0	3.2	8.8	4.3	38	—	0
F.T.	622671	Bronchogenic carcinoma with bone metastases	11–10–52	15.1	3.2	4.1	2.1	59	6.6	4.1	2.5	11.6	3.7	43	—	+++
J.C.[b]	524037	Sarcoidosis	5–11–50	13.7	4.1	—	1.8	49	7.4	—	—	9.5[c]	4.6[c]	44[c]	25.0	+++
J.C.[b]	524037	Sarcoidosis	11–7–50	13.4	4.5	—	—	67	7.4	—	—	9.3	5.1[c]	47[c]	30.0	+
M.J.	575693	Vitamin D intoxication	7–18–51	10.3	6.2	—	1.9	138	7.6	4.9	2.7	7.0	7.1[c]	49[c]	—	++++
H.T.	604014	Parathyroid adenoma	9–15–53	12.2	5.4	27.1	—	98	5.1	3.1	2.0	8.3[d]	6.1[d]	51	25.0	0
H.D.	657728	Multiple myeloma	11–27–53	14.0	5.5	7.3	2.4	54	6.5	4.4	2.1	9.9	5.5	54	25.0	++++
C.S.	363301	Carcinoma of breast with bone metastases	6–5–53	17.3	4.7	7.2	1.6	83	7.0	4.5	2.5	11.5	5.0	58	35.0	++++

[a] The figures in this column represent the lowest Ca x P products of control inorganic solutions in which cartilage from the same rat calcified.

[b] Serum not sterile.

[c] Ultrafiltration studies made on serum drawn from same patient on a different date. The values thus obtained for percentage of ultrafilterable Ca & P were used in determining the values for ultrafilterable Ca & P in the serum used for in vitro calcification.

[d] Sera from these patients never ultrafiltered. Ultrafilterable Ca calculated as 68% total serum Ca. Ultrafilterable P calculated as 113% total serum inorganic P.

magnesium was over 4.0 mg. per 100 ml. and the mean for the 18 cases was 3.6 mg. per 100 ml.

For approximately one-half the sera studied, control experiments were performed with inorganic calcifying solutions using slices obtained from the same rachitic rat. In these experiments, the lowest Ca × P products at which calcification occurred varied between 22.5 and 37.5 but was usually between 25.0 and 30.0. In six experiments (Table II, cases, C. R., L. W., E. W. and L. K.; Table III, cases J. E. and H. T.) in which sera with adequate Ca × P products failed to calcify rachitic rat cartilage, such control studies were performed. In none of these instances could that failure be ascribed to reduced calcifiability of the cartilage.

DISCUSSION

From these studies it would appear that sera obtained from patients who are not azotemic will nearly always calcify rachitic rat cartilage, if the Ca × P product of the serum ultrafiltrate, as determined by the Lavietes technique, is 34 or more. There were two exceptions to this statement in which the Ca × P products were 36 and 37. Control experiments gave no indication that the calcifiability of the cartilage incubated in the latter serum (Table I, case J. H.) was reduced. Control experiments were not performed in the former case (Table I—pooled adult serum A). However, in view of the slight difference in the calcifiability of rachitic cartilage from rat to rat and a certain unavoidable error associated with calcium and phosphorus determinations, it is not surprising that there should be some variation in the minimum Ca × P product necessary for calcification. It is felt, therefore, that little significance can be attached to the failure of calcification to occur in the two sera with products of 36 and 37.

The relationship of the hypothetical simple ion product Ca × P to the presence or absence of healing in rickets has been recognized since the early investigations of Howland (1). He noted that when the Ca × P product* of the serum of children was below 30, rickets was invariably present; and that, when it was above 40, either demonstrable healing was taking place or there had never been any rickets. With products between 30 and 40, rickets was usually present. Studies on calcification of rachitic rat cartilage in vitro, using inorganic solutions, have also disclosed a similar relationship. Calcification usually occurs with a Ca × P product approximating 30 when the concentration of other electrolytes is physiological.

Of the sera listed in Tables I and III, we were particularly interested in those

* Howland's Ca × P products are not comparable to those of this communication. He used values for *total* serum Ca × P in calculating Ca × P products, whereas we have used the levels in the serum ultrafiltrates. Furthermore, he discusses healing of rickets in human infants, whereas our data are obtained using cartilage from rachitic *rats*. Higher Ca × P products are necessary to heal rickets in the rat than in the human.

obtained from patients who were intoxicated by vitamin D or dihydrotachysterol. Rats poisoned with massive doses of vitamin D (100,000 to 125,000 daily for 7 to 9 days) have wide osteoid borders in which no lime salts are detectable by the silver nitrate technique (20, 21). Although wide osteoid borders are also found in rickets, the picture seen in these rats differs somewhat from that of ordinary rickets in that there is relatively little change in the epiphyseal cartilage, which remains thin. The cause of the wide osteoid borders in vitamin D poisoning is unknown.* There are at least three possible explanations. Perhaps new osteoid is being laid down at such a rapid rate that lime salt deposition cannot keep pace. A second possibility is that, under the influence of vitamin D, bone which has already been calcified is decalcified with the resultant formation of wide osteoid borders. If such were the case, this would be the only known biological halisteresis—i.e., lime salts being removed from calcified skeletal matrix without the latter being destroyed as well (22). A third possibility is that there is some substance in the sera of D poisoned animals which prevents calcification in osteoid as it is being formed, in spite of a more than adequate concentration of calcium and phosphorus in the serum. The absence of a wide band of uncalcified epiphyseal cartilage is not entirely against the latter hypothesis since the long bones of these animals were much shorter than those of control animals, indicating that cartilage growth was depressed. If the last mentioned possibility were the case, the ability of sera from D intoxicated patients or animals to calcify rachitic cartilage in vitro should be decreased. However, the results obtained with the four such sera studied indicate that they behaved no differently from the rest of the group. Serum from one patient with hypoparathyroidism and vitamin D poisoning (Table I, case M. F. #534975) was obtained on two different occasions. When the Ca × P product of the serum ultrafiltrate was only 31, no calcification occurred, whereas when it was 39, calcification did occur. Calcification also took place in the serum of a patient who had hypoparathyroidism and dihydrotachysterol intoxication (Table I—M. F. #639718) when the Ca × P product of the ultrafiltrate was 37. Serum from a third patient (Table III—M. J.) who was recovering from vitamin D poisoning, produced calcification with an ultrafiltrate Ca × P product of 49 in spite of an elevation of the blood non-protein nitrogen to 138 mg. per 100 ml. We have also studied pooled serum obtained from rats poisoned with a total dose of 1,050,000 units of vitamin D, each over a 7-day period, and have found it to calcify rachitic rat *cartilage* in vitro. The total calcium concentra-

* It is quite possible that such changes do not occur in the human at all. The doses of vitamin D used in these experimental studies were much greater than those taken by humans with D poisoning. Furthermore, the vitamin D was administered to young rats in which osteoid is normally being rapidly formed. To the best of our knowledge, wide osteoid borders have as yet not been reported in humans intoxicated with vitamin D, although reports of pathologic findings in bone obtained from such cases are rare.

tion in this serum was 15.4 mg. per 100 ml. and the phosphorus 8.5 mg. per 100 ml. Ultrafiltration studies were not performed on this serum but have been performed on the serum of other rats similarly poisoned with vitamin D. In these studies, 68% of the total serum calcium and 97% of the total serum phosphorus was found to be ultrafilterable. Using these percentage figures, the calculated Ca × P product of the serum used for in vitro calcification is 87. Since the serum of vitamin D poisoned rats is able to calcify the cartilage of rachitic rats, it is difficult to believe that the absence of lime salts in the osteoid of vitamin D poisoned animals is due to some factor in the serum which is inhibitory to calcification—unless totally different mechanisms are responsible for the calcification of cartilage and osteoid. However, Follis was able to calcify the osteoid from vitamin D poisoned animals in vitro by immersing the tissue in normal rat serum (23). The results of these experiments indicate, therefore, that the osteoid borders in the vitamin D poisoned rat result from a different mechanism than that which induces the osteoid seen in chronic renal insufficiency.

Another of the major reasons for undertaking this study was to see if the sera of patients who have chronic renal insufficiency is deficient in its calcifying ability. The results show that such indeed is the case because with sera obtained from these patients, definitely higher concentrations of calcium and phosphorus were necessary to calcify rachitic rat cartilage. Usually calcification did not occur unless the Ca × P product of the ultrafiltrate was 55 or more, and in one case it did not occur when this product was 87. At the present time we are unable to adequately explain the different behavior of this group of sera. However, there are a number of ways in which these sera obviously differ from normal, and the possible etiologic role of these factors will be briefly discussed.

It is unlikely that an elevated concentration of urea in the serum affects its calcifying ability. In their experiments on "in vitro" calcification, Robison and Rosenheim (24) found that the addition of urea in concentrations up to 60 mg. per 100 ml. to inorganic calcifying solutions had no inhibitory effect upon calcification. We have extended these experiments by adding urea in concentrations of 100 mg. and 200 mg. per 100 ml. to inorganic solutions and have found no change in the minimal Ca × P product required to produce calcification. The effects of adding creatinine and uric acid have not been studied.

Serum sodium and chloride concentrations were not greatly altered in any of these sera and often were normal (Table II). Even if these values had been greatly lowered, it is improbable that the ability of the sera to calcify rachitic cartilage would be adversely affected because Shipley and Holt (25) found that the presence of sodium chloride in inorganic solutions actually had an inhibitory effect upon calcification. Robison, McLeod and Rosenheim (6) confirmed these observations. They found that in the absence of sodium chloride, calcification occurred in inorganic solutions with the unusually low concentrations of cal-

cium and inorganic phosphorus of 8 mg. per 100 ml. and 2.5 mg. per 100 ml., respectively. When the concentrations of calcium and phosphorus were 8 mg. per 100 ml. and 3.0 mg. per 100 ml. respectively, calcification was obtained when the sodium chloride content was only 0.2% but was inhibited when it was 0.5%.

Robison and his coworkers (6) also investigated the effect of decreasing the sodium bicarbonate upon in vitro calcification. If they drastically lowered the sodium bicarbonate content of inorganic solutions from 0.2%, which is the amount normally present in plasma, to 0.03%, definitely higher Ca × P products were necessary for calcification. When the bicarbonate was omitted entirely, still higher Ca × P products were required. However, this degree of reduction of the sodium bicarbonate content is considerably greater than the bicarbonate reduction of the nephritic sera studied in this report. In only three of our cases was the CO_2 combining power reduced to less than one-half of normal (Table II). We have performed experiments with inorganic solutions, in which the sodium bicarbonate content was reduced to one-half the amount normally present in serum. This reduction produced little if any inhibition of calcification so long as the pH of the solutions was maintained between 7.2 and 7.6. Another indication that lowered bicarbonate content is probably not responsible for the decreased calcifying ability of nephritic sera is the fact that some sera which failed to calcify with Ca × P products of over 50 had normal CO_2 combining powers (Table II—cases E. W. and G. B.).

The inhibitory effect of magnesium upon in vitro calcification has been known for many years. Shipley and Holt (17) and later Kramer, Shipley and Orent (18) found that magnesium in very low concentrations (less than 1 mM per litre) retarded calcification in inorganic solutions. Robison, McLeod and Rosenheim (6) confirmed these results. They found that 2 mg. magnesium per 100 ml., which is the amount normally present in serum, had an adverse effect upon calcification and with twice this concentration, inhibition was pronounced. Rosenheim (19) showed that the same effect was exerted in inorganic solutions with added protein. In view of these experiments, the finding that the serum magnesium was elevated above 2.8 mg. per 100 ml. in all but five of the cases with chronic renal insufficiency and at the upper limits of normal in the remainder is of great interest. In some instances the magnesium content of the serum was three to four times the amount normally present. Ultrafilterable magnesium was not determined. However, Hopkins, Howard and Eisenberg (4) found the serum magnesium to be approximately 83% ultrafilterable when the serum proteins were normal, and hence in many of these cases the magnesium which was not "bound" to protein was still in the range where a moderate inhibitory effect might be expected. It would seem entirely possible that the decreased ability of many of these sera to calcify rachitic rat cartilage was due in part to an increased magnesium content. It is unlikely, however, that magnesium is the

sole or even the major factor concerned. In considering the individual cases in Table II, one cannot predict on the basis of magnesium levels which sera would or would not produce calcification. For example, serum from B. W. which produced calcification with the relatively low Ca × P product of 41, had a fairly high magnesium content of 4.4 mg. per 100 ml. On the other hand, sera from M. F., J. M. and L. W. with Ca × P products of 39, 42 and 45 did not produce calcification even though the serum magnesium in these cases was just at the upper limits of normal.

At the present time, therefore, the chief factor or factors responsible for the decreased ability of sera from patients with chronic renal insufficiency to calcify rachitic rat cartilage are unidentified, although an elevated serum magnesium may play a role.

Whatever the cause, the findings are of interest because of the bone disease which is so often associated with chronic renal insufficiency. There was a period during which most authorities have considered this osseous involvement to be of similar microscopic appearance to the osteitis fibrosa of hyperparathyroidism, but have attributed these changes to the chronic acidosis induced by the renal insufficiency rather than to the secondary hyperparathyroidism which may also be present (26). Follis and Jackson (27) have pointed out, however, that osteitis fibrosa is often not the predominant bone change in chronic renal insufficiency. In approximately one-half of 39 adult cases which they studied, a pathological picture similar to that seen in osteomalacia or adult rickets was present. Only one-third of the series presented lesions of osteitis fibrosa. In a given case, osteomalacia or osteitis fibrosa might exist singly or together. No correlation was found between the degree of bone change and either the clinical duration of the renal disease or the levels of the serum chemical constituents. Follis (28) later reported similar findings in a series of 16 cases of renal insufficiency in children and adolescents. He again emphasized that "renal rickets" is often truly rickets, despite previous opinion to the contrary. In one of the cases in this series, wide osteoid borders were present without there being any evidence of acidosis. This would indicate that chronic acidosis is certainly not the only factor involved in the production of these lesions. Follis suggested that "derangements in the calcium-phosphorus concentrations lead to the production of a medium which does not have the power to calcify osteoid and cartilage". The findings herein reported suggest that there is indeed some abnormality of the serum of patients with chronic renal insufficiency which interferes with its ability to calcify the cartilage of the rachitic rat even though the content of calcium and phosphorus in such serum is greater than that ordinarily required for calcification. If these same findings are applicable to human cartilage and osteoid, they would offer an explanation for this type of skeletal abnormality of renal rickets.

The proportion of the total serum calcium which is present and physiologi-

cally available in the extracellular fluid is still a matter of uncertainty. McLean and Hastings (3), using the frog heart method, obtained data which suggested that roughly one-half the total serum calcium is in ionic form and hence would pass the capillary membrane, whereas with the Lavietes technique employed in this study, approximately 70% of the total serum calcium is ultrafilterable (4, 10). One of the objectives of the present study was to see if data derived from in vitro calcification studies would throw any light on whether extracellular fluid normally contains 50% or 70% as much calcium as does the serum. The findings suggest that the proportion of the serum calcium which is biologically active, at least so far as the deposition of skeletal lime salts is concerned, actually lies somewhere *between* 50% and 70% of the total. This statement is based upon the following reasoning. In the majority of our experiments, rachitic rat cartilage underwent calcification in inorganic solutions having the Ca × P products of 25.0 to 30.0. Therefore, calcification should also have occurred in sera when their ultrafiltrates had similar Ca × P products, if all the ultrafilterable calcium were biologically active. However, calcification in serum never occurred until the Ca × P product of the ultrafiltrate was at least 34. This suggests that our ultrafiltrate method gives figures too high to reflect accurately the amount of calcium in the extracellular fluid in vivo—i.e., capillary filtration. On the other hand, certain of the data indicate that values for biologically active calcium calculated from McLean and Hastings' nomogram (3) are slightly too low. The Ca × P products in the extracellular fluids of cases A. M., E. P., C. K. and B. G. (Table I) were recalculated using calcium values obtained from this nomogram instead of values for ultrafilterable calcium but using the same phosphorus values. The Ca × P products obtained on such a basis are given in Table IV and in each instance are actually less than the lowest Ca × P products which calcified cartilage from the same rat (as used to study the serum in each case) in the control experiments using inorganic solutions.

TABLE IV

Results of In Vitro Calcification of Rachitic Rat Cartilage, Using Human Serum, Comparing Ca × P Products Obtained by Ultrafiltration Technique with Those Derived from Nomogram of McLean and Hastings (3)

Patient	Serum			Ultrafiltrate			*Nomogram			Lowest Ca × P product producing calcification in control inorganic solutions	Results (Calcification)
	Ca mg.%	P mg.%	T.P. gm.%	Ca mg.%	P mg.%	Ca × P	*Ca mg.%	P mg.%	Ca × P		
A.M.	8.4	5.5	7.6	5.7	5.9	34	3.5	5.9	21	35.0	+++
E.P.	9.9	4.8	7.1	6.9	5.3	36	4.2	5.3	22	25.0	+++
C.K.	13.9	3.5	7.2	9.7	3.9	38	6.3	3.9	25	30.0	+++
B.G.	9.9	4.7	7.1	7.2	5.5	39	4.4	5.5	24	27.5	+

* Ca^{++} determined from McLean and Hastings nomogram.

SUMMARY AND CONCLUSIONS

1. Fifty-eight human sera have been subjected to ultrafiltration studies and have been tested for their ability to calcify rachitic rat cartilage. These sera were obtained from normal persons and from those in whom, for various reasons, either the serum calcium or phosphorus was elevated.

2. The sera from patients without azotemia nearly always produced calcification when the product of the calcium expressed as mg. per 100 ml. × the concentration of the phosphorus expressed as mg. per 100 ml. in the serum ultrafiltrate, was 34 or more.

3. Sera obtained from humans and rats intoxicated with vitamin D did not appear deficient in its ability to calcify rachitic rat cartilage.

4. Sera obtained from patients with chronic renal insufficiency usually did not produce calcification until the Ca × P product of the ultrafiltrate was 55 or more.

5. Some of the factors which might theoretically play a role in the decreased calcifying ability of sera from patients with renal insufficiency have been discussed. These include retention of urea, lowered serum sodium or chloride, lowered serum bicarbonate and an elevated serum magnesium. It is concluded that, of these factors, only the magnesium concentration might possibly play a causative role and that at present the chief factors are unidentified.

6. The possible relationship of the above findings to the pathogenesis of renal rickets is discussed.

7. The data herein presented suggest that the concentration of biologically active calcium in extracellular fluid in calcification mechanisms lies between 50% and 70% of the *total* calcium concentration of the serum.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Mr. Harry Eisenberg for his assistance and helpful technical advice and to Mrs. Beverly Willen who performed most of the chemical analyses.

REFERENCES

1. Howland, J.: Etiology and pathogenesis of rickets. Medicine, 1923, **2:** 349.
2. a. Shipley, P. G.: The healing of rickety bones in vitro. Bull. Johns Hopkins Hosp., 1924, **35:** 304. b. Shipley, P. G., Kramer, B. and Howland, J.: Calcification of rachitic bones in vitro. Am. J. Dis. Child., 1925, **30:** 37.
3. McLean, F. C. and Hastings, A. B.: Clinical estimations and significance of calcium ion concentrations in the blood. Am. J. M. Sc., 1935, **189:** 601.
4. Hopkins, T., Howard, J. E. and Eisenberg, H.: Ultrafiltration studies on calcium and phosphorus in human serum. Bull. Johns Hopkins Hosp., 1952, **91:** 1.
5. Shipley, P. G., Kramer, B. and Howland, J.: Studies upon calcification in vitro. Biochem. J., 1926, **20:** 379.
6. Robison, R., McLeod, M. and Rosenheim, A. H.: The possible significance of hexosephosphoric esters in ossification. IX. Calcification in vitro. Biochem. J., 1930, **24:** 1927.

7. GUTMAN, A. B. AND YU, T. F.: Further studies on the relation between glycogenolysis and calcification in cartilage. *Metabolic Interrelations. Transactions of the First Conference.* Josiah Macy, Jr. Foundation, New York, 1949, p. 11.
8. ROBISON, R. AND SOEMES, K. R.: The possible significance of hexosephosphoric esters in calcification. VIII. Calcification in vitro. Biochem. J., 1930, **24:** 1922.
9. LAVIETES, P. H.: Anaerobic ultrafiltration. J. Biol. Chem., 1937, **120:** 267.
10. HOPKINS, T. R., CONNOR, T. B., AND HOWARD, J. E.: Ultrafiltration studies on calcium and phosphorus in pathological human serum. Bull. Johns Hopkins Hosp., 1953, **93:** 249.
11. ROSENHEIM, A. H.: Variability in the activity of the calcifying mechanism in the bones of rachitic rats. Biochem. J., 1934, **28:** 708.
12. CLARK, E. P. AND COLLIP, J. B.: A study of the Tisdall method for the determinations of blood serum calcium with a suggested modification. J. Biol. Chem., 1925, **63:** 461.
13. UMBREIT, W. W., BURRIS, R. H. AND STAUFFER, J. F.: *Manometric Techniques and Related Methods for the Study of Tissue Metabolism.* Burgess Publishing Co., Minneapolis, Minn., 1945.
14. FISKE, C. H. AND SUBBAROW, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., 1925, **66:** 375.
15. BODANSKY, A.: Phosphatase studies. II. Determination of serum phosphatase. Factors influencing accuracy of determination. J. Biol. Chem., 1933, **101:** 93.
16. DENIS, W.: The determination of magnesium in blood, plasma and serum. J. Biol. Chem., 1922, **52:** 411.
17. SHIPLEY, P. G. AND HOLT, L. E., JR.: Studies on calcification in vitro. The effect of inorganic salts. Bull. Johns Hopkins Hosp., 1927, **41:** 437.
18. KRAMER, B., SHELLING, D. H. AND ORENT, E. R.: Studies upon calcification in vitro. II. On the inhibitory effect of the magnesium ion. Bull. Johns Hopkins Hosp., 1927, **41:** 426.
19. ROSENHEIM, A. H.: The effect of serum proteins on calcification in vitro. Biochem. J., 1934, **28:** 699.
20. HAM, A. W. AND LEWIS, M. D.: Hypervitaminosis D rickets. The action of vitamin D. Brit. J. Exper. Path., 1934, **15:** 228.
21. FOLLIS, R. H., JR.: The influence of essential nutrients and hormones on cartilage and bone. *Metabolic Interrelations. Transactions of the Second Conference.* Josiah Macy, Jr. Foundation, New York, 1950, p. 221.
22. PARK, E. A.: Bone growth in health and disease. The Leonard Parsons Memorial Lectures I and II. Birmingham, England, 1953.
23. FOLLIS, R. H., JR.: Personal communication.
24. ROBISON, R. AND ROSENHEIM, A. H.: Calcification of hypertrophic cartilage in vitro. Biochem. J., 1934, **28:** 684.
25. SHIPLEY, P. G. AND HOLT, L. E., JR.: The effect of inorganic salts on calcification. Bull. Johns Hopkins Hosp., 1927, **40:** 1.
26. ALBRIGHT, F. AND REIFENSTEIN, E. C., JR.: *The Parathyroid Glands and Metabolic Bone Disease.* The Williams & Wilkins Co., Baltimore, 1948, pp. 115–116.
27. FOLLIS, R. H., JR. AND JACKSON, D. B.: Renal osteomalacia and osteitis fibrosa in adults. Bull. Johns Hopkins Hosp., 1943, **72:** 232.
28. FOLLIS, R. H., JR.: Renal rickets and osteitis fibrosa in children and adolescents. Bull. Johns Hopkins Hosp., 1950, **87:** 593.

THE EFFECT OF SODIUM PYRUVATE ON THE METABOLISM OF ETHYL ALCOHOL IN DOGS

PHILIP WHITTLESEY[1]

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine

Received for publication September 27, 1954

It has been shown by Westerfeld and his associates (1) that the rate-limiting reaction in the metabolism of alcohol is the step from alcohol to acetaldehyde. Among the agents used in an attempt to accelerate this step, pyruvic acid has been conspicuous. There has not been general agreement, however, among investigators in their interpretation of results obtained with this agent *in vivo*. Westerfeld, Stotz and Berg (1, 2) and Greenberg (3) have reported that the rate of disappearance of alcohol is accelerated following the administration of pyruvate to dogs; Gregory, Ewing, Duff-White and Thomas (4) Hulpieu, Cole and Smolenski (5), and Kinard, McCord and Aull (6) report no effect on the rate, while Bartlett and Barnet (7) and Vitale, Hegsted and Zamcheck (8) report that the rate of metabolism of alcohol is actually inhibited by pyruvate. The present study is a report of the effect of sodium pyruvate on the serum concentrations of alcohol in a group of dogs with and without pentobarbital anesthesia for which control rates of disappearance of alcohol from the serum had been established (9). It is shown that on the whole pyruvate above a given dose level does increase the rate of fall of alcohol from the serum. The observed rates of fall of serum alcohol with pyruvate and pentobarbital generally are increased above control rates with pentobarbital alone.

METHODS

The same four female mongrel dogs were used on which control studies of rates of fall of alcohol in the serum with and without pentobarbital anesthesia were previously reported (9). The care and treatment of the animals and conditions of the experiments were unchanged except for the administration of pyruvate or alanine and fluid. The method of chemical determination of alcohol was again that of Bücher and Redetzki (10). When used, heat was supplied by two GE PS8A2 heating pads as before. Performance of experiments, including dose and dilution of alcohol, doses of pentobarbital, drawing of samples and time factors were unchanged. Pyruvate was found not to interfere with the determination of alcohol.

In the latter two-thirds of the present study urine was obtained by catheterization for the four-hour period of observation. Duplicate determinations of alcohol were made on the pooled specimen. Normal urine gave readings of 1–3 mg. per cent as alcohol. The urinary excretion of alcohol is reported as the maximum average possible loss of alcohol in milligrams per 100 ml. serum per hour which could be accounted for by urinary excretion. Thus, a 15 kg. dog with a volume of distribution for alcohol of 66 per cent body weight excreting

[1] Eli Lilly Fellow in Pharmacology and Experimental Therapeutics.

200 ml. urine over the four-hour collection period containing 50 mg. per cent alcohol would have an average loss of 25 mg./hour alcohol in the urine. This could account for a maximum average loss from the volume of distribution or serum of 0.25 mg. per cent/hour and would be reported in this form.

Also in the same latter experiments the triplicate samples of each serum specimen were read in the Beckman spectrophotometer by random selection using permutation tables. Comparison of results of both methods of reading samples as measured by $s^2_{0/3}$, the variance of triplicate sample readings about their mean for five specimens in each experiment, by the "F test" (15) showed no significant difference between them.

Repetition of control rates of alcohol disappearance from the serum run at intervals during the study showed the same range of values as initial control study rates previously reported (9).

The sodium pyruvate or *dl*-(α)-alanine were given in the molar quantities indicated in the tables by gavage in 10 ml./kg. water at hourly intervals beginning 15 minutes before drawing the "one hour specimen" of blood. When given intravenously the hourly doses of pyruvate and fluid were unchanged from the oral doses and were delivered as a constant infusion through a Bowman pump. The crystalline sodium pyruvate was obtained from three different sources and was dissolved and diluted just before administration.

RESULTS

The data obtained from 24 experiments on four dogs with and without anesthesia, using the usual 1 gm./kg. alcohol intravenously and 5mM sodium pyruvate/kg./hr. are summarized in Table I. The rates of disappearance of alcohol from the serum determined from the data in Table I together with the errors of the determinations for each experiment are shown in Table II with the control rates and errors of determination (9) for comparison. In the experiments without anesthesia the straight-line regression slopes of the serum alcohol, $\hat{b}$, show an increase for the dogs as a group over their control levels of about 18 per cent (Velva 23.5, Kabish 12.5, Ara 6.2, Dirne 29.6 per cent). This figure has been depressed by inclusion of the rates of fall of serum alcohol in Ara who showed about the same range of values with pyruvate as her control rates. The experiments with pentobarbital showed an increase for the dogs as a group in the rate of fall of serum alcohol, $\hat{b}$, of about 22 per cent over their control rates (Velva 34.8, Kabish 21.6, Ara −3.0, Dirne 36.1 per cent). Except for Ara the rates during the administration of pyruvate were approximately the same with or without pentobarbital, while the control rates with pentobarbital were lower than without it. The presence of the added pyruvate did not alter the requirements of pentobarbital for the maintenance of anesthesia from those necessary during the control studies. The deviation of the hourly values about the straight-line regression slopes showed no significant difference from control figures either with or without anesthesia. Tendency toward curvature of the regression slope was about the same with as without anesthesia and in neither case was strikingly evident. Urine volumes were several fold the normal expectation of 50–75 ml. observed during the four-hour control study periods when

TABLE I

Data on Serum Alcohol Concentrations following the Intravenous Administration of Ethyl Alcohol

Dog and weight	Expt. no.	Hourly average serum alcohol concentrations in mg.%					Urine alcohol conc. in mg.%	Maximum average serum alc. loss via urine mg.%/hr.
		1	2	3	4	5		
Sodium Pyruvate[a], without Pentobarbital								
Velva	18	146.6	121.6	101.2	80.1	58.5		
kg.	21	147.8	130.3	108.1	87.4	66.7		
14.1–17.8	33	128.4	103.4	82.1	57.8	41.5	75	1.23
	36[b]	141.9	118.5	98.9	84.6	64.6	104	0.73
Kabish	11	122.1	102.5	82.9	63.4	48.6	79	0.64
kg.	14	132.3	108.0	84.4	66.2	50.7	89	0.79
17.4–18.4	19	122.6	101.0	82.9	60.7	45.7	81	0.79
	16[b]	123.5	104.4	90.0	72.3	61.7	98	0.70
Ara	12	150.9	126.9	111.1	87.7	69.3	96	0.79
kg.	14	139.5	117.9	98.3	76.0	57.2	93	0.78
12.8–14.5	19	156.3	130.5	103.9	81.8	59.8	87	0.46
	16[b]	142.0	124.5	105.6	90.7	71.6	112	1.37
Dirne	13	159.0	132.6	106.5	79.7	55.5		
kg.	17	138.0	109.7	81.1	51.1	21.1		
12.8–15.6	27	125.9	101.5	72.0	43.7	18.1	64	0.68
	29[b]	136.8	111.9	88.4	70.8	53.7	89	0.49
Sodium Pyruvate[a], with Pentobarbital								
Velva	17	130.1	107.1	84.6	66.9	44.6		
kg.	20	138.4	113.6	93.9	74.4	55.3		
14.1–17.8	29	126.7	106.3	86.2	68.3	48.6		
	35[d]	180.5	160.8	140.6	121.0	97.6	135	1.49
	14[e]	158.0	129.8	105.6	76.3	52.3		
	22[e]	152.8	130.7	105.8	83.2	62.0		
Kabish	12	119.4	102.5	89.1	67.4	46.5	66	0.39
kg.	15	124.6	102.2	88.0	65.5	45.1	72	0.65
17.4–18.4	21	152.1	130.3	113.6	90.0	70.3	107	1.87
	20[d]	124.5	106.0	91.4	72.4	52.0	87	1.90
	17[b]	131.5	111.9	97.7	83.3	73.0	102	0.70
Ara	13	122.7	106.1	87.8	72.8	58.9	62	0.39
kg.	15	141.3	123.3	103.8	89.4	71.6	93	0.76
12.8–14.5	21	148.6	118.9	96.6	75.9	59.8	95	0.62
	20[d]	170.5	151.3	130.2	109.0	91.3	123	1.68
	17[b]	147.5	122.9	100.8	81.6	64.3	100	0.55
Dirne	11	144.0	116.9	90.7	65.7	40.5		
kg.	15	151.7	124.4	105.8	82.7	60.1		
12.8–15.6	23	145.2	121.9	97.1	69.0	45.3		
	30[d]	149.5	121.7	93.4	70.6	46.8	98	2.48
	14[e]	158.1	134.4	103.2	77.6	55.5		
	18[e]	152.9	131.9	100.1	72.2	44.0		

TABLE I. *Continued*

Dog and weight	Expt. no.	Hourly average serum alcohol concentrations in mg.%					Urine alcohol conc. in mg.%	Maximum average serum alc. loss via urine mg.%/hr.
		1	2	3	4	5		
Alanine,[a] without Pentobarbital								
Velva	27	142.2	113.9	86.0	56.9	27.0		
kg.	28[b]	143.2	122.1	101.6	83.2	57.9		
15.6–16.6	34[c]	116.3	92.4	71.1	48.4	26.9	69	0.17
Kabish	13[c]	131.5	110.0	89.9	70.9	53.3	101	1.15
kg.	18[b]	140.4	119.1	98.6	77.8	61.0	116	2.01
17.4–19.2	23	135.9	109.3	81.6	60.4	34.2	92	1.25
Ara	18[b]	122.9	91.8	68.8	46.0	21.2	83	1.31
kg.	22	159.5	142.0	121.5	94.1	76.6	94	0.50
14.2–15.3								
Dirne	28[b]	145.8	117.6	92.9	68.9	44.0	92	0.90
kg.	32	168.6	138.9	110.0	85.1	59.0	117	1.00
14.6								

[a] Administration orally of 5 mM/kg./hr. except as indicated in column "Expt. No."
[b] 2.5 mM/kg./hr.
[c] 1.25 mM/kg./hr.
[d] Intravenously.
[e] Heating pads used. (See Text)

no additional fluids were given. No striking difference was observed between the urine volumes obtained with and without anesthesia. Control four-hour urinary losses of alcohol in these dogs using 1 gm./kg. alcohol showed a maximum average possible loss from the body as reflected in the serum alcohol values, through urinary excretion, of 0.1–0.2 mg. per cent/hr. The urinary losses in those experiments in which they were determined are given in Table I. The increase in rate of fall of serum alcohol through increased urinary excretion, though many fold the normal alcohol excretion, is unable to account for the increased values of $\hat{b}$ over control values. Respiratory rates were not noticeably altered from control rates making any increased loss of alcohol from the respiratory tract unlikely.

The experience with all the dogs, but particularly Kabish and Ara, indicated that the dose of 5mM/kg./hr. of pyruvate was close to the maximum tolerated dose. Vomiting and/or watery diarrhea, both with and without anesthesia, was occasionally observed. Since this disturbance might well interfere with absorption of the pyruvate and mask any effect of the metabolism of alcohol, six experiments were carried out using 2.5 mM/kg./hr. pyruvate. The results of these studies are shown in Tables I and II. The slopes, $\hat{b}$, with or without anesthesia are of the same range as control values. The volume of urine re-

TABLE II

Rate of Fall of Serum Alcohol following the Administration of Ethyl Alcohol[a]

Velva			Kabish			Ara			Dirne		
Expt. no.	b mg%/hr	$s^2_{b/3}$ mg%[2]	Expt. no.	b mg%/hr	$s^2_{b/3}$ mg%[2]	Expt. no.	b mg%/hr	$s^2_{b/3}$ mg%[2]	Expt. no.	b mg%/hr	$s^2_{b/3}$ mg%[2]
Without Pentobarbital											
Control											
1	17.6	0.588	2	19.4	0.901	1	18.2	0.252	1	21.5	0.248
3	17.0	0.264	4	16.4	0.660	3	19.0	0.054	3	25.5	0.311
5	17.9	0.270	6	15.1	0.802	8	22.5	1.017	5	18.6	0.516
7	19.2	0.521	8	18.0	0.493	9	24.3	1.212	8	20.2	0.535
9	14.9	1.550	10	17.9	1.265	10	18.1	1.196	10	20.3	0.688
Na pyruvate 5 mM/kg/hr											
18	21.8	1.154	11	18.6	1.380	12	20.2	0.822	13	26.0	1.591
21	20.5	0.984	14	20.5	0.662	14	20.7	1.106	17	29.2	0.333
33	22.0	0.787	19	19.4	1.136	19	24.2	0.711	27	27.3	0.492
Na pyruvate 2.5 mM/kg/hr											
36	18.9	0.677	16	15.6	0.394	16	17.5	2.586	29	20.7	0.882
dl-(α)-Alanine[e]											
27	28.7	1.005	13[c]	19.6	0.047	18[b]	24.9	0.159	28[b]	25.2	1.454
28[b]	21.0	0.261	18[b]	20.0	0.642	22	21.4	0.910	32	27.3	0.922
34[c]	22.3	0.319	23	25.2	0.476						
With Pentobarbital											
Control											
2	15.1	0.231	1	15.7	1.182	2	16.2	0.279	2	19.6	0.495
4	14.8	1.063	3	15.5	0.235	4	22.6	1.340	4	18.5	1.988
6	14.5	0.254	5	15.5	0.164	5	17.3	1.146	6	16.9	0.752
8	16.3	0.447	7	14.7	1.490	6	19.4	3.305	7	16.4	0.501
10	14.8	0.343	9	18.2	0.721	7	19.8	0.391	9	18.8	1.773
15[c]	15.6	1.248							12[c]	19.6	0.493
19[c]	15.8	0.878							16[c]	19.4	0.924
Na pyruvate 5 mM/kg/hr											
17	21.1	1.298	12	18.1	2.033	13	16.1	0.389	11	25.8	1.655
20	20.5	0.759	15	19.6	0.550	15	17.3	0.411	15	22.5	0.713
29	19.4	0.784	21	20.4	0.444	21	22.1	0.532	23	25.3	0.689
35[d]	20.6	0.841	20[d]	17.9	0.251	17[b]	20.8	1.310	30[d]	25.6	0.563
14[c]	26.5	0.484	17[b]	14.6	1.384	20[d]	20.1	0.575	14[c]	26.2	0.626
22[c]	22.9	0.660							18[c]	27.8	0.724

[a] Inclusive of excretory losses.

[b] Administration orally of 5 mM/kg./hr. except as indicated in column "Expt. No."

[b] 2.5 mM/kg./hr. [c] 1.25 mM/kg./hr. [d] Intravenously.

[e] Heating pads used. (See Text).

b = rate of decrease of serum alcohol, *i.e.* slope. It is estimated from the equation

$$b = \frac{-\sum_{i=1}^{5} (x_i - \bar{x})(y_i - \bar{y})}{\sum_{i=1}^{5} (x_i - \bar{x})^2}$$

where x_i denotes hourly intervals, y_i the mean hourly serum alcohol concentrations in mg./hr.

$s^2_{b/3}$ = error of chemical determinations. It is estimated from the formula

$$s^2_0 = \frac{\sum_{i=1}^{5} \sum_{j=1}^{3} (z_{ij} - y_i)^2}{5 \times 2}$$

where z_{ij} denotes individual aliquot serum alcohol values in mg.%, y_i the average value of three aliquots of a single serum specimen, 5 the number of specimens, and 2 the degrees of freedom in each group of aliquots.

covered was of the same order of magnitude as during the studies with 5 mM/kg./hr. pyruvate.

Since Kabish and Ara, particularly, had shown intestinal disturbance from 5 mM/kg./hr. pyruvate and had shown little or no change in $\hat{b}$ at either 2.5 or 5 mM/kg./hr. pyruvate by mouth, the pyruvate was given as a constant intravenous infusion with anesthesia at 5 mM/kg./hr. with ammonium chloride 2.5 mM/kg./hr. in 10 ml./kg./hr. water to obviate possible lack of absorption. The ammonium chloride was added to provide chloride, initial studies without ammonium chloride having shown the animals to have rapid respirations and a picture similar to alkalotic tetany. The data obtained are presented in Tables I and II. Dirne and Velva showed the same increase in $\hat{b}$ as observed with pyruvate by gavage. Neither Kabish nor Ara showed any increase in $\hat{b}$ over their control rate without pyruvate. Respirations were moderately increased in all dogs during the infusion.

Four experiments as shown in Tables I and II were carried out under anesthesia with pyruvate 5 mM/kg./hr. using body heating pads to maintain the rectal temperature at pre-anesthetic levels. Under this circumstance the slopes, $\hat{b}$, were increased and even slightly exceeded the $\hat{b}$'s observed with pyruvate and anesthesia where no additional heat was used.

The possibility that alanine, which is capable of rapid conversion to pyruvate in the body, might have effects comparable to pyruvate on the rate of fall of serum alcohol was investigated. Ten experiments were done without anesthesia and are summarized in Tables I and II. The *dl*-(α)-alanine was given in 1.25, 2.5 or 5 mM/kg./hr. by gavage in 10 ml./kg./hr. water. Velva, Kabish and Dirne showed increases in $\hat{b}$ at least at the 5 mM/kg./hr. dosage level equal to or greater than the rates obtained with pyruvate 5 mM/kg./hr. It is of interest that Ara showed with alanine, as with pyruvate, no change in $\hat{b}$ even at 5 mM/kg./hr. over control values. Signs of intestinal disturbance were entirely absent in this group of experiments. Urinary losses of alcohol were equal to or slightly greater than the losses observed with pyruvate.

DISCUSSION

Certain features of the present study would appear to account for some of the diversity of conclusions previously drawn. Pyruvate at doses of 2.5 mK/kg./hr. showed no effect on the rate of fall of serum alcohol. Even at 5 mM/kg./hr. pyruvate had no effect in one of the dogs studied. Since this was found to be about the maximum tolerated dose, Greenberg's dose of 9.1 mM/kg. seems quite remarkable. For purposes of comparison the doses and recorded rates found by other workers are shown in Table III. Since initial studies showed the duration of effect of pyruvate to be between one and two hours, the rates set forth in Table III are those taken from the figures or charts of various authors for a period of 1–2 hours after the initial administration of pyruvate.

TABLE III

Comparison of Rates of Fall of Blood Alcohol following Administration of Alcohol and Sodium Pyruvate in Dogs Found by Other Workers

Investigator	Route, dose & interval of Na pyruvate mM/kg.				Control rate of blood alc. mg%/hr.	Rate of fall of blood alc. for 1-2 hrs. after admin. of Na pyruvate	Remarks
	Dose	No. of Doses	Interval min.	Route			
Westerfeld et al.	6.5	2	90	p.o.	6.6	21.8	Control rates determined for each dog just before pyruvate was given.
	3.34	2	60	p.o.	8.2	21.2	
	4.6	2	60	p.o.	8.1	25.2	
	4.13	1		p.o.	7.7	11.3	
	3.27	2	60	p.o.	8.4	16.0	
	3.7	2	60	p.o.	9.5	25.3	
Greenberg	9.1	1		i.v.	20	44	"
	9.1	1		i.v.	21	36	
	9.1	1		p.o.	24	29	
	9.1	1		p.o.	22	34	
Hulpieu et al.	45.45*	3	90	i.v.	13	20	"Control" expts. run 1 week before final experiment.
	3.85	3	90	i.v.	11	12	
	2.74	3	90	i.v.	9	1.5	
	45.45*	3	90	i.v.	15	12	
	3.89	2	60	p.o.	18.3	11	Control rates determined for each dog just before pyruvate was given
	6.06	2	60	p.o.	15.3	32	
	4.79	2	60	p.o.	23.7	27	
	5.94	2	60	p.o.	21	23	
	45.45*	1		i.v.	17.5	3.5	
	2.62	1		i.v.	26.0	17.0	
	91.00*	1		i.v.	12.5	3.5	
	91.00*	1		i.v.	21.0	7.5	
Gregory et al.	4.55	2	60	p.o.	14	22	"
	4.55	2	45	i.v.	21	25	
Kinard et al.	No utilizable data						

*Total dose. No weight given.

Under this condition it is clear that most workers have obtained increased rates of disappearance of blood alcohol during a period of one to two hours after the administration of pyruvate. The striking range of rates of decrease of blood alcohol recorded by Hulpieu *et al.* (5) for both control and pyruvate experiments makes their date difficult to interpret. The demonstrated (11, 9) variability of the normal rate of decrease of serum alcohol from hour to hour and from day to day in the same as well as different animals makes it difficult, however, to evaluate any except large increases in rate of disappearance of serum alcohol without the presence of proper control studies. Bartlett (7) and Vitale (8) using radio-active alcohol in *rats* recorded an inhibition of metabolism of alcohol when accompanied by pyruvate based on the assumption that the expired $C^{14}O_2$ was

an adequate indicator of the metabolism of alcohol. They recorded a decrease in the amount of $C^{14}O_2$ in the expired air following the administration of pyruvate with the alcohol as compared to control recoveries, which they interpret as a diminution in the rate of metabolism of alcohol. Their data, however, do not preclude the possibility that an increased percentage of the metabolised alcohol was being used in synthetic reactions over a short period of time rather than being more rapidly oxidized to carbon dioxide and water as was found in the studies not involving additional pyruvate. It would be of interest to know the effects of comparable studies, in addition to the determination of blood alcohol, in dogs.

With regard to the studies of the effect of alanine on the rate of alcohol metabolism, Widmark's work in dogs (12, 13) suggested, and Eggleton (14) in cats and Westerfeld (2) in dogs recorded increases in rate of disappearance of blood alcohol uniformly with doses equal to or greater than those used in this study. It is assumed that the *dl*-racemic mixture was used in all these studies. It is to be noted that the alanine is equi-effective in altering the rate of fall of serum alcohol in quantities equi-molar with effective doses of pyruvate. This would suggest that either the *dextro* isomer is just as effectively utilized as the *levo* isomer or, if the *dextro* isomer is not utilized, that the *levo* isomer is twice as effective as pyruvate in equi-molar concentrations in altering the rate of fall of serum alcohol. The latter possibility would seem improbable.

The maximal rates obtained in this study for all dogs were between 20–30 mg. per cent/hr. decrease of alcohol in the serum when either pyruvate or alanine was administered in addition to the alcohol. Each animal appears to have its own maximal metabolic rate for alcohol. The further addition of pentobarbital appears to have little effect in the presence of pyruvate or alanine though it generally decreased the rates of alcohol disappearance from the blood in the absence of pyruvate or alanine. The percentage increase in the rate of disappearance of alcohol was thus greater in the presence of pentobarbital than in its absence. The greatest percentage increases in rate with pyruvate reported in the literature (Westerfeld *et al.*, 1, 2) resulted where the control rates were low although the maximal rates were in the range observed by several others.

SUMMARY

The effect of sodium pyruvate on the rate of disappearance of administered alcohol from the serum in dogs with and without anesthesia has been studied. Following administration of pyruvate 5 mM/kg./hr. the rates were increased for dogs as a group without anesthesia by an average of 18 per cent above control rates. Rates during pentobarbital anesthesia were increased following administration of pyruvate by an average of 22 per cent over control rates with anesthesia.

Administration of *dl*-(α)-alanine without anesthesia in doses equi-molar with

effective doses of pyruvate was followed by increases in the rate of fall of serum alcohol equal to or greater than those observed with pyruvate.

One dog of the group showed no response to either pyruvate or alanine in doses which produced definite responses in the other animals.

ADDENDUM

Since this paper was submitted for publication Vitale, Hegsted, McGrath, Grable and Zamcheck (16) have presented data indicating that sodium pyruvate in the *rat* depresses the rate of fall of blood alcohol about 30 mg. per cent per hour for a one hour period of observation after administration of the pyruvate. This is interpreted as meaning that the influences of pyruvate on alcohol metabolism in the rat and dog are not comparable. Repetition of these studies in the dog by these workers would be of interest.

REFERENCES

1. Westerfeld, W. W., Stotz, E. and Berg, R. L.: The coupled oxidation-reduction of alcohol and pyruvate in vivo. J. Biol. Chem., 1943, **149:** 237.
2. Westerfeld, W. W., Stotz, E. and Berg, R. L.: The role of pyruvate in the metabolism of ethyl alcohol. J. Biol. Chem., 1942, **144:** 657.
3. Greenberg, L. A.: Acetoin not a product of alcohol metabolism. Quart. J. Stud. Alcohol, 1942, **3:** 347.
4. Gregory, R., Ewing, P. L., Duff-White, V. and Thomas, D.: The effect of sodium pyruvate on the rate of metabolism of ethyl alcohol. Proc. Soc. Exp. Biol. and Med., 1943, **54:** 209.
5. Hulpieu, H. R., Cole, V. V. and Smolenski, U.: The failure of pyruvate and arsenite to alter alcohol metabolism. Quart. J. Stud. Alcohol, 1948, **8:** 553.
6. Kinard, F. W., McCord, W. M. and Aull, J. C., Jr.: The failure of oxygen, oxygen-carbon dioxide, or pyruvate to alter alcohol metabolism. Quart J. Stud. Alcohol, 1951, **12:** 179.
7. Bartlett, G. R. and Barnet, H. N.: Some observations on alcohol metabolism with radioactive ethyl alcohol. Quart. J. Stud. Alcohol, 1949, **10:** 381.
8. Vitale, J. J., Hegsted, D. M. and Zamcheck, N.: Effect of acetate and pyruvate on alcohol metabolism. Fed. Proc., 1954, **13:** 481.
9. Whittlesey, P.: The effects of pentobarbital on the metabolism of ethyl alcohol in dogs. Bull. Johns Hopkins Hosp., 1954, **95:** 81.
10. Bücher, T. und Redetzki, H.: Eine spezifische photometrische Bestimmung von Äthylalkohol auf fermentativem Wege. Klin. Woch., 1951, **29:** 615.
11. Marshall, E. K., Jr. and Fritz, W. F.: The metabolism of ethyl alcohol. J. Pharmacol. and Exp. Therap., 1953, **109:** 431.
12. Widmark, E. M. P.: Über die Einwirkung von Aminosäuren auf den Alkoholgehalt des Blutes. Biochem. Z., 1933, **265:** 237.
13. Widmark, E. M. P.: Studien über den Einfluss verschiedener Nahrungsbestandteile auf den Äthylalkoholgehalt des Blutes. Biochem. Z., 1934, **270:** 297.
14. Eggleton, M. G.: Some factors affecting the metabolic rate of alcohol. J. Physiol., 1940, **98:** 239.
15. Fisher, R. A.: *Statistical Methods for Research Workers*. Oliver and Boyd, Edinburgh, 1941.
16. Vitale, J. J., Hegsted, D. M., McGrath, H., Grable, E. and Zamcheck, N.: The effect of acetate, pyruvate, and glucose on alcohol metabolism. J. Biol. Chem., 1954, **210:** 753.

DR. HAMMAN AS I KNEW HIM*

CHARLES W. WAINWRIGHT

Received for publication September 28, 1954

In attempting to present a picture of the Dr. Louis Hamman I knew my regret is that I am so utterly incapable of giving expression to my true feelings about him. What I have to say about Dr. Hamman is based not alone upon my observation of him from the student's point of view, nor on my contact with him as a house officer, nor even upon a closer contact with him as a member of the Hospital staff, but upon more than twenty years of intimate personal and professional relationship with him in the practice of medicine. I admit at the outset that I am prejudiced, but if your regard for him is any less than mine, I am sure it is because my intimacy with him for so long permitted my affection and my esteem to become the greater because of this closer contact. During such a relationship you come to know a man rather well, and when you see the traits of character you admire never vary in the face of trials, and the charm of personality maintained though the circumstances may be trying, your regard becomes enthusiasm, and you soon find yourself making an idol of such a person. You come to know what he is likely to think, what his reaction to a given situation would be, and I continue to ask myself when decisions are difficult,—what would Dr. Hamman do under these circumstances? When I can picture that clearly and act upon it, I have far greater confidence in the correctness of my action, for he was a clear and logical thinker, as guileless a man as I ever knew, and possessed of that rarest of human qualities,—a charity toward the views and opinions of others that was beautiful to behold. He never was too busy to listen and had that happy faculty of making you feel that your problem was his own. You left him with advice and counsel that you felt was based on real understanding and that his conclusions were yours.

Dr. Hamman was a native of Baltimore, born in 1877; and he pursued his preliminary education in the schools of Baltimore and at Rock Hill College, then in Ellicott City, but now extinct. He did not enter upon a career in medicine lightly nor did he come from a long line of physicians. As a young man he entertained the idea of entering the priesthood. At that time he read extensively in theology and he abandoned such a connection with the Church only after serious consideration. Having made his decision, he matriculated in the Johns Hopkins Medical School, pursued the study of medicine with enthusiasm, and took his degree in 1901. He went to New York for his hospital training as a member of the resident staff of the New York Hospital. Completing this, he returned to Baltimore to engage in the practice of medicine and immediately

* Read before the Johns Hopkins Medical History Club, March 3, 1954.

became actively connected with the medical department of the Johns Hopkins Medical School as a part-time member of the staff. He pursued medicine with his full vigor and soon his penetrating and analytical mind was evident to Dr. Osler and others under whom he worked. During his earlier medical life he was much interested in pulmonary tuberculosis. With Dr. Wolman and Dr. Bordman, and later Dr. Krause and Dr. Austrian, he was quite active in the Phipps Tuberculosis Clinic. His writing at this time dealt largely with tuberculosis. His volume written with Dr. Wolman, "Tuberculin in Diagnosis and Treatment" (1) was an exhaustive clinical study of the subject. He had no desire to be known as a lung specialist, and I think later directed his attention and his writing to the circulation and other fields of interest to escape, in part at least, being so labeled. His intersts were wide and varied and by no means as specialized as the tendency is today. When asked upon one occasion if he were a lung specialist, he was heard to remark he was not a specialist in anything. During this period he was active in dispensary teaching, whereas his later activities were confined largely to clinics and bedside teaching in the wards. For the last fifteen years of his life he presented the clinical discussions of the weekly clinical-pathological conferences which he loved and so much enjoyed. Here his analytical and diagnostic acumen had unhampered range. For many years, and at the time of his death, he was an associate professor in the department of medicine, and for a year during the First World War, he was the acting head of the department. His academic activities were always on a part-time basis and even when in charge of the department, he carried on his practice with his office in the hospital. He was not too happy in this, not because he did not enjoy it for he loved teaching, but the magnitude of the task, attempting to do both, was too great, and he was not content with anything short of finished and polished work.

Dr. Hamman was a thin, even undernourished appearing man, of average height but agile and with a tremendous capacity for work. No matter how hard the day, any new problem commanded his interest and attention. He moved rapidly but with grace, and many of you can recall his long, swinging stride as he moved down the corridor of the Hospital, intent upon the matter at hand. He was easily distracted from his thoughts by the approach of a friend, to whom his greeting was warm and cordial, or by the familiar face of a student that had become fixed upon his mind by some incident in one of his clinics, and his greeting was apt to be with some reference to that incident. Dr. Blalock told me only recently that Dr. Hamman greeted him for years as *the* authority on purpura simplex. This was because of the fact that Dr. Blalock, as a third year student, had expected to present a case of purpura simplex in Dr. Hamman's clinic and had read everything he could find on the subject. He never presented the case but in some way Dr. Hamman had heard of this extensive preparation, and for a long time thereafter he always greeted our pro-

fessor of surgery as *the* authority on purpura simplex. As much as he enjoyed this play, it never was his desire to embarrass you and he derived no pleasure from putting you in an awkward position if you were unable to defend yourself, but he did prefer it to be at the expense of the other fellow. He was indeed, quite sensitive and I think could best be described as "thin skinned." If he ever exceeded bounds with his barbs and thrusts, it was his effort to stimulate repartee which was exciting to him and genuine fun.

I dare say few could think of Dr. Hamman as an athlete, but in his younger days his tennis was no mean game. It may be, perhaps, even harder to think of him as a baseball player, but he has often told me of *his* estimate of himself as a second baseman. In later years auction bridge fascinated him, and whereas I am not capable of giving an estimate of his skill as a bridge player, I am sure that any one who has been his opponent will attest to his delight in successfully completing a contract. All through his life he was a prodigious reader, his literary interests were wide and varied, and often one would find him, before leaving his office for the weekend, carefully perusing the Saturday Review of Literature, a periodical he constantly read. In later years he found his recreation and diversion almost wholly in reading.

Dr. Hamman was youthful in appearance, and with his quickness of movement, gave the impression of being a man much younger than his years. He prided himself on his youthful appearance. When someone spoke of how young he looked, he would beam, and he often has turned to me later and said, "Charlie, that's my weak spot." He was conservative in his tastes as to dress and was the personification of neatness. He abhored disorder; there was a place for everything, and the orderliness of his desk made you feel that your problem was the only business at hand.

To those who studied under him, Dr. Hamman stands out as a teacher of unusual ability. Many of us recall him as a truly great teacher of medicine. When as a student one first came into contact with him, his quick thinking and his humor and wit were perhaps bewildering. His agile mind made one uncertain; many were charmed by it, whereas others were nettled, not angered, but sufficiently stimulated to feel that if work on their part would put them in a position to fend off his irritating thrusts, there should be no lack of it. When later one came to know him better and watched him perform, it was apparent that he was using this approach to impress upon you the point he wished to make. Many of his former pupils carry with them facts indelibly impressed upon their memory by some witticism of his. Seldom was he at a loss for an answer. When he gave a clinical-pathological conference with Dr. Rich and made the wrong diagnosis, Dr. Rich, after demonstrating the true state of affairs, would ask Dr. Hamman if he had any further comment to make. He would not be abashed but would smile and say, "Well, Dr. Rich, this case only goes to show that the best diagnosis is not always the correct diagnosis." Who could resist

such a man or fail to be charmed by his ability to rise to such an occasion. The only time I can recall a situation that was too much for him was in one of his third-year clinics. He was trying to make a student tell him why the patient had swelling of one leg. The student was struggling, and after giving him every reason but the right one, told Dr. Hamman, as a dying gasp, that he thought the patient had a nephritis. When Dr. Hamman remonstrated and told the student he was sure both legs would be swollen if the swelling was due to nephritis, the student replied he thought *it was a unilateral nephritis*. This was too much for him, he threw up his hands, utterly unable to go on.

Dr. Hamman was possessed of an unusual ability to detect the essence of a clinical problem, and it was inspiring to follow his logical reasoning which gave voice to his extensive clinical experience. His ability to sift the wheat from the chaff, to separate the important from the unimportant data, made his conclusions seem *so* natural that it was quite impossible to arrive at any other diagnosis. What was even more important was his ability to think aloud and to carry you with him, so as to make this intricate process of arriving at the correct diagnosis seem simple and easy. With this capacity, one can readily understand that in a clinical-pathological conference he would be at his best. Indeed, he was a master of this form of medical teaching, whom few could equal and none excel. His discussions were forthright and his conclusions unequivocal, and he was never one to make apologies for faulty reasoning. He often said to me when his conclusions were incorrect, "Charlie, seldom right but never undecided." His teaching was a source of great pleasure to him, and as he approached the statutory age for academic retirement, it troubled him for he always felt that time was well spent when he could give students of his rich clinical experience. I am sure I do not exaggerate when I say that many considered it a privilege to attend his exercises, for few left his presence without carrying with them some fundamental truth deeply impressed upon them. Dr. Hamman loved youth, its enthusiasm, its eagerness, its boldness, and he was never one to stifle it. However, he did feel it his duty to stress fundamental truths and to clarify the thinking of youth. He was in no sense a showman, but modest and dignified in his bearing. He felt that medicine was a high calling and at all times, particularly when he represented his profession before the students, he felt it incumbent upon him to maintain that dignity and to instill in them the importance of commanding the respect to which he felt medicine was entitled. He never was stuffy about it but did it with ease and grace, and as effective as his methods were, his witticisms in no way lessened the students' respect for him, but rather enhanced their regard and esteem to a degree that few enjoy.

Dr. Hamman was fond of attending medical meetings although the opportunity to do so was less in his later years. Not only did these give him the

opportunity to hear new ideas and conclusions presented, but it was a time when he could have the social intercourse of his friends, and they were many. He enjoyed extemporaneous discussion, and in those earlier years Dr. Hamman and his close friend, Dr. Paullin, of Atlanta, enjoyed nothing more than to sit on the front row at a meeting of the "Southern Medical" or the "Climatological", and to discuss many of the subjects presented, to the point that he was embarrassed at being on his feet so much. He refrained to a large extent in later years, but perhaps it was the technical nature of presentations that deterred him rather than any lack of desire to exchange views. He was conservative rather than venturesome in his approach to medicine, but was never one to reject new ideas because they were new. He did, however, insist that they be proved of worth. At such gatherings he was an active participant in after-dinner discussions, and his friends and colleagues revelled in the active play of his mind. Their estimate of his capacity, his brilliance, and his position in American medicine are indicated in his selection in 1941 as president of the Association of American Physicians. The establishment of a scholarship in the Johns Hopkins Medical School in his memory by his associates and students was an expression of their esteem and high regard.

Dr. Hamman was indeed a student of clinical medicine, and he typified that rare combination of physician studying his patient in terms of pathological anatomy and physiology, but with a humanity that caused him never to forget that the disease which interested him so much was part and parcel of the human being before him. He was always conscious of the fact that the patient possessed the human emotions common to us all, and that individuals, sick and well, were such vastly different persons. In his manner he was kind and gentle; he was quiet and unassuming; never austere, but considerate; always striving to put his patient at ease, and at the same time creating a confidence which inspired those to whom he ministered to a renewed hope. Few patients failed to be helped by consulting him. On many occasions his schedule was completely disrupted because he felt it necessary to give his patient full opportunity to talk out his troubles, and his concern was always the welfare of his patient, both mental and physical. He was by no means a nihilist but used drugs sparingly and with a specificity that would naturally limit their use. In the functional disorder he was ready always to try to get at the true cause of the disturbance, explain the symptoms it produced and really help one to help oneself.

Dr. Hamman's literary contributions to medicine are largely upon clinical subjects. He was a stickler for precise and correct English and clarity of expression. I think the tone of International Clinics during his editorship will attest to this fact. In later years a philosophical vein tended to permeate his writing. It was an approach he enjoyed, but he had no desire to become

an elder statesman. He was always actively contributing. His banquet address before the Association of American Physicians "As Others See Us" (2) was perhaps the most satisfying outlet for this philosophical trend of his thought. Many of his writings have become classics of medicine, not because of their originality but because of his accurate judgment of values and the orderliness with which he stressed their importance. Searching in his thinking and intringued by the mystifying, he was constantly in quest of the reason for the features of clinical medicine which had hitherto defied explanation. His description of mediastinal emphysema was the culmination of his search for the explanation of a most unusual and baffling noise he had heard in a chest.

Until the day of his death he displayed his usual mental and physical vigor, and it can be said of him that he died at his zenith. He would have had it thus, for he was deeply concerned and spoke with dread, to those close to him, of passing over the crest and continuing beyond the peak. To have his life brought to a close by sudden death was his wish, and he has indicated in his writings that man's greatest blessing in life is "sudden death." (3). His wish was gratified and on April 28, 1946, midway in his sixty ninth year, a coronary artery thrombosis brought his useful life to a close. Those to whom he ministered lost a considerate and understanding doctor; those who knew him well, a wise counsellor and gentle friend; and our profession surrendered a brilliant exponent of sound clinical medicine, who walked humbly, and a truly great physician.

BIBLIOGRAPHY

1. HAMMAN, L. AND WOLMAN, S.: *Tuberculin in Diagnosis and Treatment*. D. Appleton & Co., New York, N. Y., 1912.
2. HAMMAN, L.: As others see us. Tr. Assn. Am. Physn. 1938, **53:** 22.
3. HAMMAN, L.: Sudden death. Bull. Johns Hopkins Hosp., 1934, **55:** 387.

SICKLEMIA AND INFARCTION OF THE SPLEEN DURING AERIAL FLIGHT

Electrophoresis of the Hemoglobin in 15 Cases

ERNEST W. SMITH* AND C. LOCKARD CONLEY

The Department of Medicine, The Johns Hopkins University School of Medicine and Hospital

Received for publication October 5, 1954

The degree of sickling of the erythrocytes of individuals with sicklemia is well known to be related to the oxygen tension to which the blood is exposed. Lower oxygen tension is required to produce the sickling phenomenon in sickle cell trait than in sickle cell anemia (1, 2). Presumably the degree of hypoxia necessary for inducing sickling is a function of the concentration of the abnormal S (sickle) hemoglobin in the red cell. It has generally been assumed that the various manifestations of sickle cell disease are attributable to the consequences of intravascular sickling. Supportive evidence for this concept is provided by the demonstrated absence of sickling *in vivo* in the sickle cell trait and the frequent finding at autopsy of occlusions of small blood vessels by masses of sickled erythrocytes in patients dying of sickle cell anemia. Theoretically, any condition which enhances sickling *in vivo* should predispose to sickle cell crisis.

These observations suggest that individuals with sicklemia might be adversely affected by exposure to reduced oxygen tension such as occurs during high altitude flight. With this concept in mind, Henderson and Thornell (3) studied the effects of simulated altitudes of 10,000 to 16,000 feet on four persons with sickle cell trait and one patient with sickle cell anemia. The periods of exposure were very brief and no untoward effects attributable to sickling were noted, although increased numbers of sickled cells were demonstrated in the blood smears of the patient with sickle cell anemia. Findley, Boulter and MacGibbon (4) observed three subjects with sickle cell trait at actual altitudes as high as 15,000 feet. Blood specimens, immediately fixed in formalin, showed no sickling at 5,000 and 10,000 feet, but blood drawn at 15,000 feet contained as many as five per cent sickled cells. No symptoms developed and there was no evidence of increased blood destruction during the brief flights. Nevertheless, these authors warned that a potential hazard existed and recommended that oxygen be available on flights in which negroes are transported.

Sullivan (5) in 1947 reported the case of an 18 year old negro soldier who developed nausea, vomiting and left upper quadrant discomfort during a flight from the east coast to California. The abdominal distress subsided after

* Clinical Fellow of the American Cancer Society.

This investigation was supported in part by a research grant of the Division of Research Grants and Fellowships, National Institutes of Health.

six days. On a return flight, when the plane reached its maximum altitude, estimated to be between 10,000 and 16,000 feet, the soldier experienced cramping pain in the left upper quadrant. The pain was subsequently aggravated by walking and jarring of the body. On examination he was found to have a greatly enlarged tender spleen, but there were no other significant physical findings. Sickling of the red cells was demonstrated and there was pronounced leucocytosis but no anemia. Symptoms subsided promptly but splenomegaly persisted during a period of observation of two months. The nature of the splenic lesion was not recognized, but Sullivan emphasized that his observation might have important implications in military medicine.

During the past 15 years many negroes have been transported by air, particularly in the military service, but until recently no other reports of sickle cell crisis occurring during flight appeared. In 1951 Diggs and Vorder Bruegge (6) at the Armed Forces Institute of Pathology studied the spleen of a negro airman who developed a crisis while at 20,000 feet. The spleen weighed 823 grams and had ruptured. Cooley and his associates (7) observed six negro soldiers who developed nausea, vomiting, pain in the left upper abdomen and fever during prolonged flights in unpressurized aircraft at altitudes between 10,000 and 15,000 feet. All of the patients had been in apparently good health prior to the flights and there was no history of previous manifestations of sickle cell disease. On examination there was pronounced left upper quadrant tenderness, paralytic ileus, fever to as high as 104° and leucocytosis. Laparotomy was performed in each case and an enlarged, grossly infarcted spleen was removed. There was no evidence of occlusion of the major splenic arteries. Sickling of the red cells was demonstrated in the peripheral blood or in the spleen in every case, but only two patients were reported to have moderately severe anemia.

The apparent rarity of infarction of the spleen during flight in individuals with sicklemia suggested to us that these patients might have had one of the genetic variants of sickle cell disease rather than sickle cell trait. By a curious coincidence, the first two cases in which electrophoretic study of the hemoglobin was performed were found to be instances of sickle cell-hemoglobin C disease (8, 9). This observation prompted us to carry out the present study in order to determine if splenic infarction during flight occurred only in the sickle cell diseases or whether it might also occur in sickle cell trait.

Since the report of Cooley and his associates (7), more than 15 additional cases of splenic infarction occurring during flight have been observed in military hospitals. Through the cooperation of the Office of the Surgeon General of the Air Force and with the assistance of many individuals, it has been possible to obtain blood for electrophoretic study from 15 persons who developed splenic infarcts during flight.

METHODS

Sterile blood specimens obtained from these 15 patients were sent to us from various parts of the world. Fourteen of the patients were negroes and one was white but was said to have somewhat dark skin. Reference is made in Table I to the individuals who contributed clinical data and made blood available to us.

Whole blood, prevented from clotting by an anticoagulant, was available from each of the 15 individuals. Filter paper electrophoresis of the hemoglobin was performed in every case by a method previously reported from this laboratory (10). Sickling tests using sodium metabisulfite were performed in those cases in which previous tests had been reported as negative. Blood smears which had been freshly prepared were available in 11 cases. Unless otherwise indicated in the text, all other hematologic data were obtained in laboratories other than our own.

RESULTS

The results of the study are summarized in Table I. Sickle (S) hemoglobin was present in the blood of each of the 15 individuals who had developed splenic infarcts during flight. In three cases C hemoglobin was also present, indicating that these were instances of sickle cell-hemoglobin C disease. The remaining 12 persons had normal (A) hemoglobin in addition to the S component. In 11 of these cases, the amount of normal hemoglobin exceeded the amount of sickle hemoglobin. This is the pattern expected in sickle cell trait. One patient (Case 4) had slightly more sickle than normal hemoglobin and about 20 per cent target cells were seen on the blood smear. A blood smear obtained from this patient's mother showed many target cells, but electrophoretic study of her hemoglobin has not been possible. It is not known whether this case is an instance of sickle cell trait or whether it may represent an unusual sickle cell-thalassemia or some other variant of sickle cell disease.

Various methods for demonstrating sickling of the red cells had been initially successful in only six of the 15 cases. In one instance, preoperative sickling tests were reported as negative but positive results were obtained after splenectomy. Sickled erythrocytes were seen in the splenic pulp even when red cells of the peripheral blood had not been observed to sickle. In five cases in which splenectomy was not performed, sickling tests had been reported as negative. However, in each of these five cases sickling tests performed in our laboratory gave unequivocally positive results.

None of the 15 individuals had been suspected of having sicklemia prior to the hospitalization for splenic infarction. Of the three patients with sickle cell-hemoglobin C disease, one had recurrent leg ulcers as a child; none had a sickle cell habitus. One of the 15 is known to have had no clinical illness during a previous air flight, while one other related that an episode of left upper abdominal pain had occurred during an earlier flight. Both of these individuals were found to have sickle cell trait.

Anemia of moderate to severe degree was encountered in 4 of the 15 patients.

TABLE I

Infarction of the Spleen in Sicklemia

(16 collected cases with hemoglobin electrophoresis)

(Cases #1–15 comprise the group studied in this laboratory)

Patient, Age, Race, Sex	Genetic Hgb Type	Previous History	Degree of Anemia	Altitude of Flight	Sickling Tests	Splenectomy	Description of Spleen	Case Source
1) G.S., 23, CM	S-C	Leg ulcers in past	RBC 2.7 mill. Hgb. 8 gm.	10–15,000 ft. approx.*	Positive	Yes	804 gm., infarction & congestion	Case #3, Cooley et al (7)
2) C.C., CM	S-C	Not significant	Ht. 35%	4–6,000 ft. (commercial)	Positive	Yes	810 gm., old & new infarction	Case # 1, Doenges et al (12)
3) N.N., CM	S-C	Not significant	Hg. 10.5 gm.	4–6,000 ft. (commercial)	Positive	No		Case #2, Doenges et al (12)
4) W.R., 23, CM	S-A	Not significant	RBC 4.6 mill. Hgb. 11.9 gm.	10–15,000 ft. approx.	Neg. pre-op pos. post-op	Yes	460 gm., massive infarction	Case # 5, Cooley et al (7)
5) L.C., 38, WM	S-A	Not significant		10–15,000 ft. approx.	Negative‡	Yes		Jernigan et al (11)
6) H.B.L., 24, CM	S-A	Not significant		10–15,000 ft. approx.	Questionable	No		Jernigan et al (11)
7) J.H., CM	S-A	Not significant	Ht. 41†	10–15,000 ft. approx.	Negative‡	No		Jernigan et al (11)
8) R.J., 22, CM	S-A	Not significant	Ht. 40†	10–15,000 ft. approx.	Positive	No		Case #1, Conn (13)
9) W.J., 22, CM	S-A	Not significant	Ht. 42†	10–15,000 ft. approx.	Positive	No		Case #2, Conn (13)
10) A.M., 22, CM	S-A	Not significant	RBC 4.5 mill. Hgb. 11.9 gm.	10–15,000 ft. approx.	Negative‡	Yes	144 gm., complete infarct	Case #1, Cooley et al (7)
11) B.H., 21, CM	S-A	Not significant	RBC 2.1 mill. Hgb. 11.9 gm.	10–15,000 ft. approx.	Negative‡	Yes	260 gm., congestion & infarction	Case #6, Cooley et al (7)
12) L.A., 25, CM	S-A	Not significant	Ht. 46 hypochromia	10–15,000 ft. approx.	Negative‡	No		Doenges (14)
13) J.J., 27, CM	S-A	Similar bout during previous flight	Ht. 44 hypochromia	10–15,000 ft. approx.	Negative‡	No		Doenges (14)
14) F.B., CM	S-A	Not significant	Ht. 38	10–15,000 ft. approx.	Negative‡	No		Doenges (14)
15) C.C., CM	S-A	Not significant			Positive	No		Wald (15)
16) CM	S-C					No		Motulsky (9)

* Figures of 10–15,000 feet are used as representative of coast-to-coast flights for military personnel returning from the Pacific.

† Hematocrit value determined following convalescence.

‡ Blood sickling preparations in our laboratory were positive.

Three of those with anemia had sickle cell-hemoglobin C disease. The fourth had sickle cell trait and while the spleen was found to be congested, it weighed only 260 grams. Thus hemorrhage into the spleen does not seem to be an acceptable explanation for the anemia. When examined following convalescence, this man was no longer anemic.

Precise altitude of flight by the planes carrying these individuals is not known to us. Jernigan (11) reported that coast-to-coast military flights returning personnel from the Pacific usually were at 10,000 to 15,000 feet and that cabins were usually not pressurized. The majority of these 15 individuals experienced splenic infarction during such flights. In two instances, however, commercial air-line flights at altitudes between 4,000 and 6,000 feet were involved. The patients who developed splenic infarcts at this low altitude were both found to have sickle cell-hemoglobin C disease.

Splenectomy was performed in 6 of the 15 cases. Fresh, extensive infarction was present in each of the spleens removed, and in one case there was evidence of an old infarct as well. The smallest spleen weighed 144 grams, and the largest 810 grams. Congestion of the splenic pulp or sub-capsular hemorrhage was frequently encountered. At least one patient who was not subjected to splenectomy had a palpably enlarged spleen.

DISCUSSION

The occurrence of splenic infarcts during flight in this rather large group of individuals with sicklemia suggests that this phenomenon is not rare. It is difficult to understand the paucity of reported cases prior to 1954, but it seems likely that in some instances the nature of the underlying disorder may not have been recognized. Of 16 patients whose hemoglobin was subjected to electrophoretic study, 4 were found to have sickle cell-hemoglobin C disease and 11 appear to have only the sickle cell trait. In the remaining case, the possibility of another genetic variant of sicklemia has not been excluded. Approximately 8 per cent of American negroes are found to have the sickle cell trait. Therefore, it seems likely that the development of splenic infarction in flight is an unusual occurrence in individuals with this inherited red cell abnormality. On the other hand, sickle cell-hemoglobin C disease is extremely uncommon, requiring the inheritance of both a gene for sickling and one for hemoglobin C. Patients with this disease often have manifestations which cause their rejection for military service. That 25 per cent of the cases studied had sickle cell-hemoglobin C disease suggests that the risk of developing a splenic infarct in flight is high in individuals with this disorder. It is noteworthy that the two patients who developed infarcts at relatively low altitudes (about 6,000 feet), had sickle cell-hemoglobin C disease. These observations indicate that flying presents a considerable hazard to persons with this combination of abnormal hemoglobins.

Of great interest is the discovery that individuals with only the sickle cell

* Figures of 10–15,000 feet are used as representative of coast-to-coast flights for military personnel returning from the Pacific.
† Hematocrit value determined following convalescence.
‡ Blood sickling preparations in our laboratory were positive.

trait may develop clinical manifestations referable to sickling. It is generally believed that the trait is entirely benign, although evidence is accumulating that in rare instances important evidences of disease may appear. Recurrent gross hematuria has been encountered in several patients demonstrated to have sickle cell trait (8). In other instances fatal diffuse disease of the brain attributable to occlusion of small blood vessels by masses of sickled cells has been encountered in patients who appeared to have only the sickle trait (16, 17). Whether individuals who develop such disorders differ in a significant way from asymptomatic individuals with sickle cell trait remains to be determined. In the present study, with one exception, there was no evidence that the patients had more S hemoglobin than is usually encountered in persons with the trait.

The mechanism by which splenic infarction is produced in these cases is not entirely clear. Intravascular sickling of erythrocytes induced by lowered oxygen tension and resulting in occlusion of small blood vessels is a logical explanation. However, it does not seem to account adequately for the episodes occurring at 6,000 feet, where the degree of reduction of atmospheric oxygen tension is slight indeed. Individuals with sicklemia apparently do not develop splenic infarcts when subjected to more severe anoxia such as may occur in pneumonia. Furthermore, it is not apparent why organs other than the spleen are not the sites of vascular occlusion during flight. The induction of a high degree of intravascular sickling does not necessarily lead to sickle cell crisis and has not been reported to cause infarction of the spleen. Administration of BAL (2,3-dimercaptopropanol) to individuals with sicklemia may cause pronounced intravascular sickling without the development of symptoms (18, 19). It seems probable that there are other factors, as yet unrecognized, in the pathogenesis of splenic infarction during aerial flight.

The discovery of these cases indicates that the sickling phenomenon may have important implications both in military and civilian aviation. It would seem that sickle cell-hemoglobin C disease should disqualify an individual from flying. There is a much smaller but definite hazard to the individual with sickle cell trait. Routine tests to detect sickling are often unreliable. Of the 15 cases in the present study 8 had been reported to have negative tests for sickling when first examined. Whenever possible, electrophoresis of the hemoglobin by a simple filter paper technic should be employed. This method is well suited for mass screening and gives reliable results.

SUMMARY

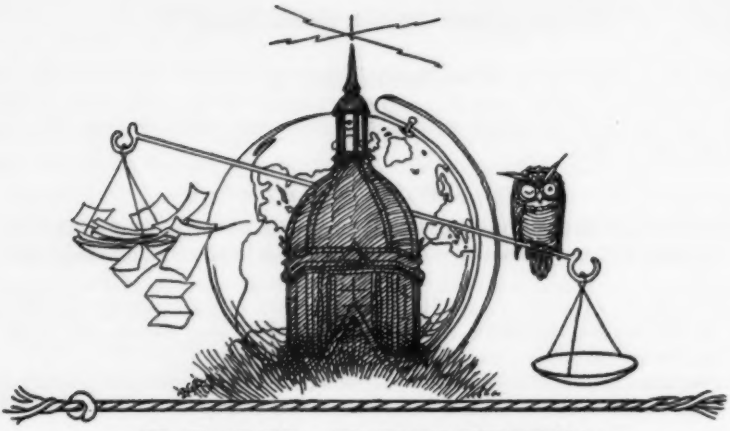
Electrophoretic analysis of the hemoglobin of 15 individuals who developed splenic infarcts during flight was performed. Sickle hemoglobin was demonstrated in all 15, and C hemoglobin in 3. Evidently flying is hazardous to persons with sickle cell-hemoglobin C disease, because of the risk of splenic infarction. Splenic infarcts may develop during flight in individuals who have only sickle cell trait.

ACKNOWLEDGMENTS

We wish to express our sincere appreciation to the many individuals whose cooperation made this study possible.

BIBLIOGRAPHY

1. Sherman, I. J.: The sickle cell phenomenon with special reference to the differentiation of sickle cell anemia from the sickle cell trait. Bull. Johns Hopkins Hosp. 1940, **67:** 309.
2. Lange, R. D., Minnich, V. and Moore, C. V.: Effect of oxygen tension and of pH on the sickling and mechanical fragility of erythrocytes from patients with sickle cell anemia and the sickle cell trait. J. Lab. and Clin. Med. 1951, **37:** 789.
3. Henderson, A. B. and Thornell, H. E.: Observations on the effect of lowered oxygen tension on sicklemia and sickle cell anemia among military flying personnel. J. Lab. and Clin. Med. 1946, **31:** 769.
4. Findlay, G. M., Boulter, E. A. and MacGibbon, C. B.: A note on sickling and flying. J. Roy. Army Med. Corps 1947, **89:** 138.
5. Sullivan, B. H., Jr.: Danger of airplane flight to persons with sicklemia. Ann. Int. Med. 1950, **32:** 338.
6. Diggs, L. W. and Vorder Brugge, C. F.: Unexpected death associated with sickle cell trait. Presented before the American Society of Clinical Pathology, Chicago, 1951, (not published).
7. Cooley, J. C., Peterson, W. L., Engel, C. E. and Jernigan, J. P.: Clinical triad of massive splenic infarction, sicklemia trait, and high altitude flying. J. Am. Med. Assn. 1954, **154:** 111.
8. Smith, E. W. and Conley, C. L.: Clinical features of the genetic variants of sickle cell disease. Bull. Johns Hopkins Hosp. 1954, **94:** 289.
9. Motulsky, A. G.: Sicklemia. J. Am. Med. Assn. 1954, **155:** 388.
10. Smith, E. W. and Conley, C. L.: Filter paper electrophoresis of human hemoglobins with special reference to the incidence and clinical significance of hemoglobin C. Bull. Johns Hopkins Hosp. 1953, **93:** 94.
11. Jernigan, J. P., Cooley, J. C., Peterson, W. L. and Engel, C. E.: Splenic infarcts associated with hypoxia. Presented before the Aero Medical Association, Washington, D. C., March, 1954.
12. Doenges, J. P., Smith, E. W., Wise, S. P. III, and Breitenbucher, R. B.: Splenic infarction following air travel and associated with the sickling phenomenon. J. Am. Med. Assn. 1954, **156:** 955.
13. Conn, H. O.: Sickle-cell trait and splenic infarction associated with high-altitude flying. New England J. Med. 1954, **251:** 417.
14. Doenges, J. P. Personal communication.
15. Wald, N. Personal communication.
16. Green, T. W. and Conley, C. L.: Occurrence of symptoms of sickle cell disease in the absence of persistent anemia. Ann. Int. Med. 1951, **34:** 849.
17. Diggs, L. W. and Jones, R. S.: Clinicopathologic conference. Am. J. Clin. Path. 1952, **22:** 1194.
18. Thomas, L. and Stetson, C. A., Jr.: Personal communication.
19. Kuhns, W. J.: Effects of the intramuscular administration of BAL (2,3 dimercaptopropanol) in a subject with the sickle cell trait: Case report. Blood 1949, **4:** 1240.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of members of the Editorial Board of this Bulletin)

William H. Welch and the Rise of Modern Medicine. By DONALD FLEMING. 216 pp., $3.00. Little Brown & Co., Boston, Massachusetts.

Although this little book is scarcely 200 pages in length, it covers in a brief but accurate form the development of medical education as we know it today. The brilliant inaugural address in 1765 by Dr. John Morgan, upon the occasion of the founding in Philadelphia of America's first medical school, enumerated nearly every important fundamental of medical education. But it was not until the turn of the twentieth century, when William H. Welch alone put most of them into a well balanced curriculum, that any significant improvement took place in the chaotic training of physicians. Welch played an all-important role not only in the founding of The Johns Hopkins Medical Institutions, but also in the development of the Rockefeller Institute, the Carnegie Institution, the National Research Council, and the American Medical Association. These activities were all factors contributing to his being regarded as the leading Influential of American science in his day.

The book begins rather slowly with the background of Welch's childhood and early schooling. It gains momentum as it unfolds the intriguing story of his years of study in New York and in Europe and of his career in Baltimore. It deals primarily with the effect which Welch's life had on the times and the times on it.

This book is highly recommended as a pleasant and informative resumé of the development of The Johns Hopkins Hospital and Medical School, and of medical education itself. Some of the problems during the early years, such as the full-time controversy, are treated in an especially interesting manner.

OSCAR M. WILBUR, JR.

Administrative Medicine. Transactions of the Second Conference December 8, 9 and 10, 1953. Edited by GEORGE S. STEVENSON. 164 pp., $3.00. The Josiah Macy, Jr. Foundation, New York, N. Y.

The Josiah Macy, Jr. Foundation presents another book consisting of the edited discussions of a three day conference on administrative medicine called by the Foundation in December 1953. This second conference considered "What is the role of administrative as distinguished from professional (or technical) personnel in organized health and medical activities? At what points is it essential to have professional (or technical) personnel? Are there circumstances in which it may be

wasteful, or even damaging, to have professional personnel?" The participants under the chairmanship of Dr. Hugh R. Leavell are recognized leaders in the field of administration as applied to medical activities in this country and represented education, hospitals, national and state public health services, nursing, health insurance programs, labor and others. A study of home-care programs presented by Dr. Alice M. Waterhouse of the Public Health Service, and a discussion of the Scottish Health Services by Sir Andrew Davidson, Chief Medical Officer, Department of Health for Scotland, served well as patterns for group consideration of the multidiscipline approach to the problems of administration in medicine and related fields.

The report maintains an atmosphere of informality that lends conviction to the expressed opinions of the conference participants whose interests and experiences are varied but whose common interest in solution of the problems involved is explicit. It is recommended to all concerned with improving methods of applying the results of modern medical and related research.

HARRY L. CHANT

Electrocardiography. By E. GREY DIMOND. 261 pp., $14.00. The C. V. Mosby Co., St. Louis, Missouri.

In the Welch Medical Library there are at present 19 textbooks on electrocardiography, all recently issued or re-edited. The fact that new books on electrocardiography continue to appear frequently in the face of such an over-supply is a phenomenon as yet unexplained. As with many of these texts, Dr. Dimond's book is the outgrowth of his experiences in teaching electrocardiography to students and graduates over a period of years. In general the organization is logical, the writing clear, and the ECG illustrations excellent. The author has, however, surrendered to the temptation to include a chapter heading on every possible subject, resulting in a seven-paragraph chapter on methods of teaching electrocardiography, ten paragraphs on the organization of an electrocardiographic department, and an extensive chapter on congenital heart disease complete with x-rays and hemodynamic diagrams which seem altogether out of place in a work of this kind. In the sections concerned with the interpretation of the electrocardiogram, this book compares favorably with other works in this field, but it cannot be said to be the best, or by any means the least expensive, of the textbooks of electrocardiography now available to the student.

WILLIAM R. MILNOR

Lectures on General Pathology. Delivered at the Sir William Dunn School of Pathology, University of Oxford May 20, 1954. Edited by SIR HOWARD FLOREY. 733 pp., $13.00. W. B. Saunders Co., Philadelphia, Pa.

This is a superb series of 37 lectures on fundamental studies in general topics of pathology delivered to advanced students at the Sir William Dunn School of Pathology at Oxford University. Sir Howard Florey is the editor of this volume and is also responsible for 10 of the lectures. Other contributors are E. P. Abraham, C. P. Gladstone, H. Harris, R. G. MacFarlane, A. H. T. Robb-Smith, K. B. Roberts and W. E. van Heyningen, all members of the School of Pathology at Oxford, G. W. Cameron of London University College Hospital Medical School, and F. K. Sanders of the London School of Hygiene and Tropical Medicine.

These authors are specialists in their fields; each contributes an exhaustive monograph on subjects of special interest. Florey's chapters cover the historical aspects of the subject of pathology and many problems concerned with inflammation—its chemical, macroscopic and microscopic changes, the cells concerned in the reaction, healing of tissue, the influence of drugs on inflammation and tuberculosis as an example of chronic inflammation. Cameron's subjects include degenerative cell and tissue changes, regeneration, hemorrhage and shock. Abraham discusses necrosis, the effects of radiant energy, antigens, antibodies and the in-vitro action of antibacterial substances. Sanders discusses tissue reaction to viruses, and with Robb-Smith, the functional significance of connective tissue. MacFarlane's subject is the reactions of the blood to injury. Wound healing is covered by Harris, and fever and edema by Roberts. Finally, Gladstone and van Heyningen cover studies in microbiology, immunity and hypersensitivity.

A historical background is given for each subject as well as a comprehensive survey of recent experimental work, stressing, especially, physiological and biochemical studies. This is no bigoted

or dogmatic presentation of gross and microscopic tissue changes as all sides of every controversial problem are included in each discussion. There are copious references to the recent literature and excellent illustrations—photographs, sketches, charts, tables and diagrams of experimental apparatus add to the presentation.

In summary, these are extraordinarily stimulating lectures and as they cover virtually all problems relating to fundamental tissue changes, this book should serve as a background not only to the advanced pathologist, but also for experimental work in all of the biological sciences. We should thank Dr. Florey for this valuable contribution to the field of pathology.

E. H. Oppenheimer

Essentials of Pediatrics, 5th edition. By P. C. Jeans, F. H. Wright and F. G. Blake. 808 pp., $4.75. J. B. Lippincott Co., Philadelphia, Pa.

It is possible that in no area of medicine are such heavy demands made upon the nurse as in the care of infants and children. First, her knowledge of medicine must be sufficient to understand a very wide range of disease, as well as to recognize and help to cope with the sudden and often alarming alterations in the state of patients' well-being which are so common in pediatrics. In addition, it is her lot to play the very important role of mother substitute. To do this she must understand the evolution of the human from newborn to adolescent so as to be aware of the needs of the child peculiar to his developmental stage. She must also be quick to recognize the needs of the particular child without much knowledge of the environment from which he came. She must fulfill these needs, both medical and emotional, with an understanding mind and a kind heart in the face of anxiety, fearfulness, rebellion or withdrawal of patients who may be suspicious or openly hostile to the hospital environment. This is a large order, and its performance is not entirely instinctive, but requires guidance.

This fifth edition of Jeans' "Essentials of Pediatrics" is such a guide, and it is a good one. In the first third of the book the authors have addressed themselves to the problems of growth and development of normal children, to the relationship of the nurse to the ill child, as well as to his parents, and to certain aspects of treatment which are peculiar to pediatrics. This, in the reviewer's opinion, is the best part of the book and it is skillfully done. It is clear that the authors have personal experience with the situations they discuss and have handled them with sympathy and good sense. The problems of admission of children to the hospital, of the need for a pleasant and happy atmosphere in the ward, of the importance of play supervision, as well as the care of seriously ill children, are discussed and well illustrated with actual incidents.

The remainder of the book, an additional 500 pages, is devoted to a systematic review of pediatric disease; its pathogenesis, symptomatology, diagnosis and treatment and, finally, a section on nutrition and feeding of infants and children. This is done clearly, concisely and with what seems adequate detail. Emphasis is laid upon those manifestions which the nurse is most likely to see and feel, leaving out detailed discussion of the refinements of physical and laboratory diagnosis. Treatment is always emphasized and, wherever applicable, there are special sections on the nursing care of the particular condition.

The material is obviously up to date. There are good sections on congenital defects of the heart, endocrine diseases, hematologic disorders, collagen diseases, and other conditions which are assuming larger proportions in hospital populations. Altogether the book is about 200 pages larger than the 4th edition.

The final section deals with feeding, nutritional requirements in health and disease, and nutritional diseases.

In general, the writing is clear and pleasing, photographs are frequent and well chosen, and the charts and diagrams are useful. This should be a valuable book for students as well as practicing pediatric nurses.

Barton Childs

The Jealous Child. By Edward Podolsky. 147 pp., $3.75. Philosophical Library, New York, N.Y.

Dr. Podolsky has described a variety of emotional and functional-structural traumata affecting

the childish personality. His cogent foreword and first chapter ably trace the general framework of life experiences and reactions as the child develops from fetus to adolescence.

Since this book is written in nontechnical language, it is apparently designed for those of widely-scattered and not necessarily professionally trained backgrounds. Parents, teachers, semi-trained and even some professionally-trained people might possibly gain some misconceptions. It is not scientific consensus that most children of 6 to 8 months are ready for mastery of bowel control, nor is it scientific consensus that dental caries have such far-reaching consequences in the personality development. Especially, it is not held (generally) that dental caries produce toxins as an etiological factor in behavior problems and personality deviations.

There are several informative chapters on such problems as rheumatic heart disease, tuberculosis, diabetes, obesity, endocrinopathies, left-handedness, speech disorders, hearing impairment and neurological disorders. Other types of maladjustment are related to broken homes, illegitimacy, parental rejection, sibling rivalry, minority group problems, and adoption.

The problems related in the above two paragraphs are generally summed up with suggestions as to preventive and corrective measures. These prescriptions tend to be repetitious and to smack of Dale Carnegieism.

Generally, the theme of jealousy is not developed. One chapter called, "The Jealous Child" is valid and especially germane to the book title. The author relates jealousy in broad terms to unfilled needs—physical, mental, emotional economic, social. Otherwise, the range of typical biological problems of developing human beings is presented in understandable terms. The bibliography is well selected and pertinent to the general emotional problems of children.

WILLIAM T. DIXON

Teaching in the Outpatient Department. By SISTER MARY ISADORE LENNON. 240 pp., $4.00 G. P. Putnam's Sons, New York, N. Y.

This book is a guide to be used in planning a sound educational program in outpatient nursing for student nurses. The author achieves her purpose by a methodical and logical presentation of the overall objectives, the organization of the outpatient department and the functions of the social service worker in the department. She discusses program planning, orientation, instructional content, related student experience, and evaluation. The program suggested is based primarily on the author's own teaching situation. However, an instructor using this book will find in it excellent suggestions for planning an interesting and stimulating program in outpatient nursing which she can adapt to her own situation.

It is regrettable that more emphasis was not given to the public health aspects of outpatient nursing or to the development of outpatient services which are not necessarily limited to the care of the indigent patient.

ELEANOR J. SMITH

Diseases of the Heart and Arteries. Anatomical and Functional Disturbances of the Circulation. Treatment. 4th edition. By GEORGE R. HERRMANN. 652 pp., $12.50. The C. V. Mosby Co., St. Louis, Missouri.

Dr. Hermann's latest edition of what he refers to as a "handbook" succeeds admirably in describing the diseases of the heart and peripheral circulation concisely, omitting the wealth of detail which is available in the larger textbooks. The presentation of so much information in a relatively small volume is accomplished in part by the liberal use of diagrams and illustrations. Many of these have been modified and redrawn from original sources, and much care has evidently been given to their selection and execution.

The approach to the subject is well suited to the undergraduate student. Chapters on the relevant aspects of the history and physical examination, and on radiological and other special techniques of study are included. Recent changes in emphasis in cardiology are reflected by inclusions of sections on embryology and congenital heart disease, antibiotic therapy, current dogmas of electrocardiography, and new concepts of the active factors in heart failure. The author's personal opinion on controversial points is always clearly apparent, while the supporting experimental evidence is, of necessity, not given in any detail. It should be pointed out that there is by no means

universal agreement on some of these subjects, for example the alleged superiority of nicotinic acid in the treatment of coronary insufficiency.

The student and the practitioner will find this a readable book, and a useful source of information.

WILLIAM R. MILNOR

Plastic Surgery at The New York Hospital One Hundred Years Ago. With Biographical Notes on Gurdon Buck. By HERBERT CONWAY AND RICHARD B. STARK. Forewords by John Hay Whitney, Stanhope Bayne-Jones and Frank Glenn. 110 pp., $5.00. Paul B. Hoeber, New York, N. Y.

Plastic Surgery at the New York Hospital One Hundred Years Ago is the story of the beginning of plastic surgery as a specialized field of medicine, in this country, the man who responded to the needs of his time, and of the institution which originated the modern concept of unity between the university, the medical school, and the hospital. This volume, while small, presents a delightful and fascinating treatment of the progress of plastic surgery in the brief span of three generations. The revolution brought about by antisepsis can be fully appreciated from the description of a pre-antisepsis surgical procedure: the instruments resting on a velvet-lined shelf; sponges which, having been washed since the previous operation, are kept in a wooden pail of fresh water; surgeon's fingers probing successively into the wounds of various patients; the horror of endemic hospital erysipelas, hospital gangrene and pyemia.

The plastic surgeon, as such, was born of an encounter between an imaginative, resourceful individual and a challenge, represented by the simultaneous crisis and stimulus of war. The man was Gurdon Buck; the crisis was the Civil War, with injuries of a type and number unknown to America until that time. The career of Gurdon Buck shows how a man with an inventive and determined mind grows by securing successive goals—from a student with a private physician to "Junior Walker" at the New York Hospital, thence to a medical house officer, visiting surgeon and, eventually, one of the most respected surgeons in the country, and the vice president of the New York Academy of Medicine.

Having established himself at the top of his profession, Gurdon Buck became a member of the Sanitary Commission, inspecting the General Hospitals of the Army during the Civil War. In this capacity he was presented with unprecedented surgical problems, the solution of which was a product of knowledge of surgical principles, plus sound analysis of the problems on hand, and meticulous performance of the procedure. These principles are still valid today. Without going into the details of his procedure, suffice to say that the field included restoration of cheek defects, congenital anomalies like macrocheilia, hairy nevus, cleft-lip, correction of webbing and scar contractures following burns, treatment of hemangiomas, and von Recklinghausen's Disease. He utilized sliding and jump flaps, and intraoral prosthesis. He emphasized the poor quality of cicatricial tissue and understood very thoroughly the principles of flap transfers. It is interesting to note that the use of free skin grafts was unknown to him, and the donor areas of the flaps were left to heal by granulation. All in all, this was a remarkable undertaking to be carried on by one man who had nothing to follow but his own judgment and imagination. In 1876, the wealth of his experiences was summarized in the first textbook of plastic surgery published in this country—"Contributions to Reparative Surgery".

An index and table of contents would be useful for the reader's orientation in this interesting and instructive book.

F. S. HOFFMEISTER

BOOKS RECEIVED FOR REVIEW

Color Atlas of Pathology, Vol. II. Prepared under the auspices of the U. S. Naval Medical School of the National Naval Medical Center, Bethesda, Md. 450 pp., $20.00. J. B. Lippincott Co., Philadelphia, Pa.

Diseases of the Skin. For Practitioners and Students, 4th edition. By GEORGE CLINTON ANDREWS. 877 pp., $13.00. W. B. Saunders Co., Philadelphia, Pa.

Harrison's Principles of Internal Medicine, 2nd edition. Edited by T. R. HARRISON, RAYMOND D. ADAMS, PAUL B. BEESON, WILLIAM H. RESNIK, GEORGE W. THORN AND M. M. WINTROBE. 1790 pp., student 1 volume edition $16.00, professional 2 volume edition $21.00. The Blakiston Co., New York, N. Y.

Meredith's Hygiene, 5th edition. By ARTHUR F. DAVIS AND WARREN H. SOUTHWORTH. 906 pp., $6.00. The Blakiston Co., New York, N. Y.

The Neuroanatomical Basis for Clinical Neurology. By TALMAGE L. PEELE. 564 pp., $12.50. McGraw-Hill Book Co., New York, N. Y.

Porphyrins. Their Biological and Chemical Importance. By A. VANNOTTI. Translated by C. Rimington. 273 pp., 50s. Hilger & Watts, London, England.

The Scourge of the Swastika. A Short History of Nazi War Crimes. By LORD RUSSELL OF LIVERPOOL. 259 pp., $4.50. Philosophical Library, New York, N. Y.

Serological Approaches to Studies of Protein Structure and Metabolism. Edited by WILLIAM H. COLE. 97 pp., $2.00. Rutgers University Press, New Brunswick, N. J.

Urology, Vols. I, II and III. Edited by MEREDITH CAMPBELL. 2,356 pp., $60.00 per set. W. B. Saunders Co., Philadelphia, Pa.